UNIVERSITY OF NAIROBI

COMPATIBILITY OF *SCHISTOSOMA MANSONI* AND ITS INTERMEDIATE HOST SNAILS (*BIOMPHALARIA SPP*) IN RELATION TO TRANSMISSION OF INTESTINAL SCHISTOSOMIASIS IN KENYA

MUTUKU MARTIN WAMBUA

SCHOOL OF BIOLOGICAL SCIENCES

I80/51031/2016

A Thesis submitted in fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Applied Parasitology of the University of Nairobi

DECLARATION

I, do hereby declare that this thesis is my original work and has not been presented for a degree or any other award in any other university.

Signature…… Date……

Martin Wambua Mutuku, Reg. No. I80/51031/2016

This thesis has been submitted for examination with our approval as university supervisors.

1. Signature…….. Date……

   Prof. Horace Ochanda

   School of Biological Sciences

   University of Nairobi, Nairobi, Kenya

2. Signature…….. Date……

   Dr. Gerald M. Mkoji

   Centre for Biotechnology Research and Development

   Kenya Medical Research Institute, Nairobi, Kenya

3. Signature…….. Date……

   Prof. Eric S. Loker

   Biology Department

   University of New Mexico, Albuquerque, USA

DEDICATION

This work is dedicated to my entire family for their unending support and encouragement throughout my study period. To my Children Mary, Joseph and John, Dad loves you. My wife, Caroline for always being there for me.

ACKNOWLEWDGEMENTS

I sincerely thank my supervisors, Prof. Horace Ochanda, School of Biological Sciences, University of Nairobi, Dr. Gerald M. Mkoji, Centre for Biotechnology Research and Development, Kenya Medical Research Institute (KEMRI), and Prof. Eric S. Loker, Department of Biology, University of New Mexico, USA for their guidance, encouragement, and mentorship during the course of this study and preparation of thesis. With their help I have gained invaluable knowledge and understanding of the subject investigated. I sincerely thank study participants who provided fecal samples from which parasite materials used in this study were isolated: Adults from Kanyibok village in Bondo, Siaya County, School children of Obuon Primary School in Asao, Kisumu county and Mukou Primary School in Mwea, Kirinyaga county. Without their cooperation and participation, this work would have been difficult to accomplish. I deeply appreciate the support and cooperation of the parents and guardians of the children for allowing their children to participate in the study, and that of teachers in the participating schools, for facilitating fecal sample collections. My sincere thanks to Dr. Phelgona Otieno of Centre for Clinical Research (CCR) KEMRI who provided clinical support to this project. Special thanks go to my colleagues in CBRD, particularly, Dr. Eric Lelo, Messrs Polycup Odongo, Boaz Oduor, Joseph Kinuthia, Geoffrey Maina, and Ibrahim Mwangi who were always available to render help. Special thanks to colleagues from Biology Department, UNM, Dr. Martina Laidemitt and Ms Caitlin Babbitt for their help in setting up the experiments. This work was undertaken with financial support from NIH Grants No. R01 AI101438, R37AI101438, D43 TW010543 and P30GM110907 and University of Georgia Research Foundation Inc., which was funded by Bill and Melinda Gates Foundation for the SCORE project. Last but not least, to the Almighty God, for giving me the strength and good health throughout the period I undertook this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

ACUC	Animal Care and Use Committee
AIDS	Acquired Immune Deficiency Syndrome
cm	Centimeters
CDC	Centers for Disease Control
CBRD	Centre for Biotechnology Research and Development
CGHR	Center for Global Health Research
CI	Confidence interval
DALYs	Disability Adjusted Life Years
DBL	Danish Bilharziasis Laboratory
FREPs	Fibrinogen Related Proteins
Glmm	Genelarised linera mixed model
GPS	Global Positioning System
HIV	Human Immunodeficiency Virus
Hr	Hours
KEMRI	Kenya Medical Research Institute
KWS	Kenya Wildlife Service
m	Meters

MDA	Mass Drug Adminstration
NACOSTI	National Commision for Science, Technology and Innovations
NEMA	National Environment Management Authority
NTDs	Neglected Tropical Diseases
PE	Post Exposure
PHS	Persistent hotspot
PZQ	Praziquantel
RESP	Responding
SCORE	Schistosomiasis Consortium for Operational Research and Evaluation
SERU	Scientific and Ethics Review Unit
SSC	Scientific Steering Committee
µM	Micrometers
UNM	University of New Mexico
WHO	World Health Organization

ABSTRACT

Introduction: Schistosomiasis afflicts an estimated 240 million people worldwide, with majority of the cases occurring in sub-Saharan Africa. In Kenya, an estimated 6 million people are infected, with >30 million people at risk of infection. *Schistosoma mansoni,* the most widespread among the human infecting *Schistosoma* species depends on the freshwater planorbid snails in the genus *Biomphalaria* for its transmission. In this study compatibility and vectorial competencies of 3 *Biomphalaria spp* were examined and snail-related factors of *S. mansoni* transmission were investigated in Lake Victoria, western Kenya

Materials and Methods: Reciprocal cross infection experiemnt using field derived *B. pfeifferi* from Kirinyaga county and *B. sudanica* from Kisumu county in 3 categories based on age/size was done using sympatric and allopatric *S. mansoni* and then, using lab-raised F1 generation of Lake Victoria derived *B. sudanica* and *B. choanomphala* snails with sympatric and allopatric combinations of *S. mansoni* in 3 different miracidia dose (1, 5 and 10). On 4 occasions over a 2-year period, relative abundance of *B. sudanica* and *B. choanomphala*, prevalence of *S. mansoni* in the snail population, hyacinth intrusion, and acquisition of *S. mansoni* worms by sentinel mice at 20 sampling sites (two per village) were determined.

Results: It was observed that *S. mansoni* developed faster and consistently had higher infection rates in *B. pfeifferi* (39.6-80.7%) than in *B. sudanica* (2.4-21.5%) regardless of the parasite source or snail size/age. Cercariae production was greater for *B. pfeifferi* as compared to B. *sudanica*. It was also observed that *B. choanomphala* was more susceptible to *S. mansoni* than *B. sudanica* (12.7 - 80.8% versus 5.2 – 18.6%) and on average, *B. choanomphala* produced more cercariae than *B. sudanica* and increase in the miracidia dose was not associated with an increased cercariae production for both snail taxa. It was also observed that shoreline-associated *B. sudanica* did not

differ in relative abundance or prevalence of *S. mansoni* infections between PHS and RESP villages ($P>0.05$). However, water hyacinth intrusions were associated with increased *B. sudanica* abundance ($P<0.05$). The deep-water *B. choanomphala*, on the other hand, was significantly more abundant in the PHS villages than in the RESP villages ($P<0.05$).

Conclusion/Recommendations: Results of this study suggest that *B. pfeifferi* is more likely to become infected by *S. mansoni* and to shed more cercariae than *B. sudanica* and *B. choanomphala*, suggesting that the risk per individual snail of perpetuating transmission in Kenyan streams or lacustrine habitats may differ considerably between these 2 habitat types. The high susceptibility of *B. choanomphala* to *S. mansoni* and its presence in all sampling sites in the PHS villages suggests that it could be providing an alternative mode of transmission that may favor greater persistence of *S. mansoni* in PHS villages. The study established evidence of transmission in all ten villages studied and thus recommends for an integrated, basin-wide control plan to counteract rapid reinfections facilitated by large snail populations and movements of infected people around Lake Victoria.

CHAPTER ONE: INTRODUCTION

1.1: Human schistosomiasis and its public health significance

Human schistosomiasis, a snail transmitted parasitic infection caused by blood flukes (also, known as schistosomes) in the genus *Schistosoma*, remains a major public health problem in the tropical regions of the world. Though majority of the world's great Neglected Tropical Diseases (NTDs) have decreased sharply in prevalence in recent years (Loker 2013), the opposite is the case for schistosomiasis with an estimated 258 million cases (WHO 2016) which is over 25% higher than estimated 50 years ago. Furthermore, the impact of schistosomiasis as estimated by disability-adjusted life years (DALYs) has been reappraised and found to be 4-30 fold higher than suggested by earlier estimates (King *et al*. 2005; Colley *et al*. 2014; King 2015). Of the world's estimated 258 million cases, 85% occur in sub-Saharan Africa (WHO 2016) and with no new data since the year 2000, it is estimated that 500-600 million more people are at risk of contracting the infection (Chitsulo *et al*. 2000). Schistosomiasis is endemic in tropical and sub-tropical regions of the world, among the poor rural communities in 78 countries in Africa, South America, Middle East and Asia (WHO 2014).

Among the schistosome species that cause human schistosomiasis, four, namely; *Schistosoma mansoni*, *S. intarcalatum*, *S. mekongi* and *S. japonicum* are responsible for causing intestinal schistosomiasis, while *S. haematobium* causes urogenital schistosomiasis. The three major schistosome species namely *S. mansoni*, *S. haematobium* and *S. japonicum* are responsible for the majority of schistosomiasis cases worldwide (WHO 2014). However, *S. mansoni* is probably the most widespread and, the most important from a public health perspective (Crompton 1999; Chitsulo *et al.* 2000). *S. mansoni* is found in many countries in Africa, South America (Brazil,

Surinam and Venezuela), the Caribbean (including Puerto Rico, St Lucia, Guadeloupe, Martinique, Dominican Republic, Antigua and Montserrat) and in parts of the Middle East (Figure 1).

In the sub-Saharan Africa, *S. mansoni* infects nearly 100 million people, mostly children and results in up to 70 million disability-adjusted life years (DALYs) lost annually, exceeding those lost due to malaria or tuberculosis and almost reaching the DALYs lost from HIV/AIDS (Crompton 1999; Hotez & Fenwick 2009). Figure 1.1 is a map showing the global distribution of schistosomiasis.

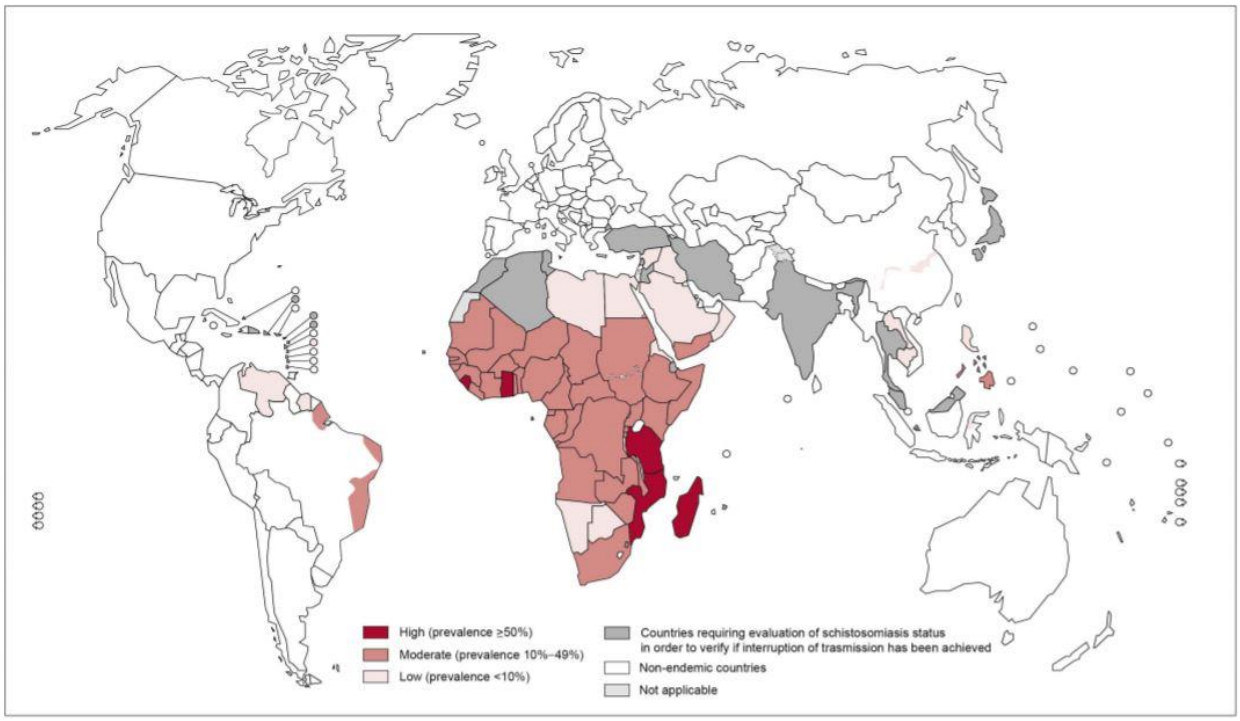


Figure 1.1: Map showing global distribution of schistosomiasis (courtesy of WHO 2011)

https://reliefweb.int/map/world/world-distribution-schistosomiasis-2011

1.2: Schistosomiasis pathogenesis

The symptoms of this disease are caused by the body's reaction to the eggs produced by the worms, not the worms themselves, and the parasite eggs that become trapped in the body tissue cause an immune reaction (WHO 2018). Intestinal schistosomiasis is a debilitating disease often

characterized by abdominal pain and discomfort, diarrhea and sometimes, blood in the stool, and in severe cases, hepatic portal hypertension (WHO 2018) and in children, it may result in impaired physical growth and cognitive ability, and a drop in physical performance (Jukes *et al*. 2002; King *et al.* 2005). Other effects of the disease include fibrotic responses to schistosome eggs trapped in the intestines, liver and other organs of the infected person (Caldas *et al*. 2008), and malnutrition especially, in children, which may retard physical growth (Ana *et al.,* 1998). Liver enlargement is common in advanced cases, and is frequently associated with accumulation of fluid in the peritoneal cavity and hypertension of the abdominal blood vessels, and in such cases, there may also be enlargement of the spleen as well (WHO 2018).

The classic sign of urogenital schistosomiasis is hematuria (blood in urine). Fibrosis of the bladder and ureter, and kidney damage is sometimes diagnosed in advanced cases. Bladder cancer is another possible complication in the later stages of urogenital schistosomaisis. In women, urogenital schistosomiasis may present with genital lesions, vaginal bleeding, pain during sexual intercourse and nodules in the vulva. In men, urogenital schistosomiasis can induce pathology of the seminal vesicles, prostate and other organs. Urogenital schistosomiasis may also, have other long-term irreversible consequences, including infertility (WHO 2018). Rarely, the parasite eggs can travel to the brain and cause seizures, paralysis and spinal cord inflammation (CDC 2008).

1.3: Transmission of intestinal schistosomiasis

Human schistosomiasis is transmitted through freshwater or amphibious gastropod snails, and in the case of *S. mansoni,* the freshwater pulmonate snails in the Family Planorbidae, and in the genus *Biomphalaria* are responsible for its transmission (Brown 2005). Several species of *Biomphalaria* are known to transmit the parasite in Africa, but the most prominent among them is *B. pfeifferi*, a

widespread inhabitant of streams and other small water bodies across sub-Saharan Africa (Brown 2005). Although by far the majority of the world's cases of *S. mansoni* occur in Africa, majority of the experimental work done on snail hosts is with *Biomphalaria glabrata* (Loker 2010), a snail restricted to the Neotropical region. Although a convenient laboratory model, *B. glabrata* supports only a minority of human schistosomiasis cases (Loker 2010). In Kenya, *S. mansoni* transmission is perpetuated by three *Biomphalaria* species 1) *B. sudanica* (an out-crossing species, mostly occurring on the shoreline of Lake Victoria and Lake Jipe; 2) *B. choanomphala* (a deep water ecophenotype of *B. sudanica* found in Lake Victoria), and 3) *B. pfeifferi* (a strong preferential self-fertilizer, usually present in streams and small water bodies across the country, except in the low lying coastal belt) (Brown 2005) . All the three species are susceptible to *S. mansoni* and contribute to the parasite transmission but may vary in compatibility, and it is hypothesized that this creates for each species unique opportunities for exploitation and control. One aspect of the parasite-snail host relationship that is not very well understood is the level of compatibility between the genetically diverse *S. mansoni* and its equally diverse snail hosts, the *Biomphalaria* spp (Theron *et al*. 2008). Compatibility of local snail populations with schistosomes is a key determinant in schistosomiasis transmission success. The greater the compatibility, the more successful the parasite will establish in the snail host and produce cercariae which are then released into the aquatic environment for transmission. One unique aspect of schistosomes is that, unlike other digenean trematodes, they have separate male and female, and the adult parasites reside in the blood stream of their definitive hosts, where they mate and produce characteristic eggs. The eggs produced by the adult female worms work their way through the host tissue and eventually find their way into the environment via excreta. In the case of *S. mansoni*, the parasite eggs find their way into the intestinal lumen, and then into the environment via stool. On coming into contact with

freshwater, the schistosome eggs hatch into motile larval forms called miracidia (singular: miracidium), which swim freely, and actively search for and enter a suitable snail host (in the case of *S. mansoni*, *Biomphalaria spp*). Following penetration into the snail host, the miracidium develops into a mother sporocyst which produe daughter sporocyst that transforms into cercariae, and in the latter, the cercariae (motile larval forms that are infective to the definitive vertebrate host are produced by asexual reproduction. The cercariae are then released into the water and swim freely in search of a suitable vertebrate. The *S. mansoni* cercariae are infective to their mammalian definitive hosts (usually humans or non-human primates such as baboons, or rodents), and enter by penetration through intact skin and transform into schistosomulae, which then migrate in the bloodstream through the lungs and then to the hepatic portal system, and eventually, to the mesenteric veins where they mature into adults and reproduce (Rollinson 1988).

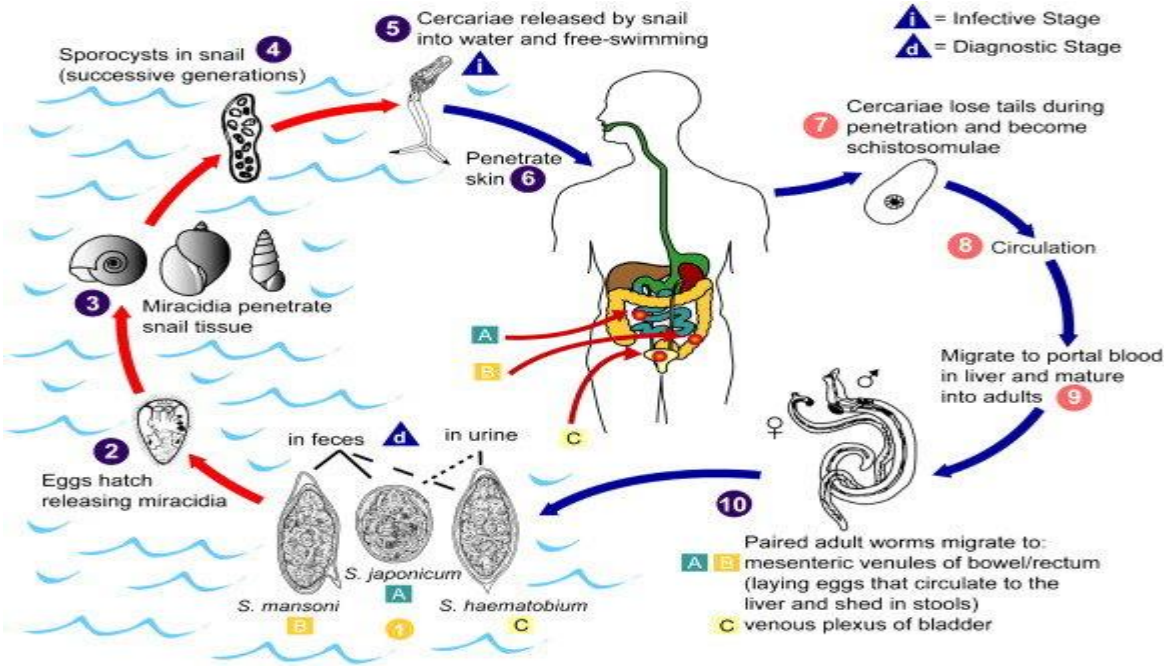


Figure 1.2: Illustration of life cycle of *Schistosoma spp.* adapted from *https://www.cdc.gov/parasites/schistosomiasis/biology.html*

1.4: Schistosomiasis in Kenya.

Schistosomiasis is endemic in Kenya with more than 6 million people infected and many more at risk of infection (Chitsulo *et al*. 2000). It is mainly found within the Mwea irrigation scheme in Kirinyaga County, Machakos, Kitui, Taita Taveta, Coastal region and counties within Lake Victoria basin in western Kenya with *S. mansoni* being the most predominant (WHO 2006). Prevalence is highest among individuals between ages 10-17 years, but adults in the rural areas, particularly those engaged in occupational activities associated with water such as irrigation farming, fishing, car washing or sand harvesting being also infected, with prevalence ranging between 5% to over 65% (Karanja *et al*. 1997).

Recent studies assessing the prevalence of *S. mansoni* infection in school children in lake Victoria basin (Odiere *et al.* 2012; Samuels *et al.* 2014) reported overall prevalence of 60.5% and 69% respectively with island areas having a 2 fold higher prevalence rates than main land villages with schools that were closest to the lakeshore having higher prevalence rates than those away from the lakeshore. This could be associated with more frequent water contact by the individuals which is potentially contaminated with cercariae because the lake and the tributaries feeding the lake are habitats for snails and so, provide a source of infection or re-infections after treatment, to the vulnerable population.

Several initiatives have been done to control schistosomiasis in western Kenya within the Lake Victoria basin, however the disease has persisted despite the combined efforts. In a more recent program supported by the Schistosomiasis Control Research and Evaluation (SCORE) implemented from 2011 - 2015 and which involved a school based annual mass drug administration (MDA) using praziquantel (PZQ) for 4 consecutive years, it was observed that prevalence amongst school children in some villages declined significantly upon treatment while

in other villages, prevalence remained the same as at the beginning of the Program or increased during the 4 annual consecutive treatments (Wiegand *et al.* 2017). In the schools that reported significant decline, initial prevalence ranged from as high as 81.6 % -52.9%, and at the end of the program in 2015, prevalence had declined to 19% - 5.5% after the annual treatment with PZQ. On the other hand, in the non-responding schools, initial prevalence which ranged from 91.8% - 60.5% remained relatively high (82% - 39%) by 2015, with one school that initially had a prevalence rate of 68.6% having an increased prevalence of 82% by the 5th year of the Program. Several factors could have contributed to this phenomenon. The present study sought to determine whether or not snail compatibility with the parasite could have played a major role in this phenomenon.

1.5: Prevention and Control

Schistosomiasis is considered among the neglected tropical parasitic infections that continue to plague the resource limited countries in tropical and sub-tropical regions of the world and which are now being targeted for elimination through a concerted global effort (WHO 2013). Current schistosomiasis control efforts rely largely on treatment of infected people with the antischistosomal drug, praziquantel (PZQ). This drug is effective, safe, and relatively low-cost. Even though re-infections may occur after treatment, the risk of developing severe disease is diminished and even reversed when treatment is initiated and repeated in childhood. This strategy focuses on reducing disease through periodic, targeted treatment through the large-scale treatment (preventive chemotherapy) of affected populations. It involves regular treatment of all at-risk individuals with an aim of reducing disease morbidity and transmission. However, this approach is difficult to sustain as re-infections frequently occur after successful treatment and is incapable on its own of interrupting transmission (King *et al*. 2006; Lelo *et al*. 2014; Secor 2014; Ross *et al*.

2017). While drug-based control efforts may reduce infection prevalence, morbidity or level of environment contamination and parasite transmission (Norton *et al*. 2010), such efforts do not completely stop transmission. Furthermore, PZQ is by no means readily available in most sub-Saharan African countries (Hotez *et al*. 2010) and therefore, sustainable control of schistosomiasis may remain problematic for a long time in many of the endemic areas. Data for 2016 show that 35.6% of people requiring treatment were reached globally, with a proportion of 53.7% of school-aged children requiring preventive chemotherapy for schistosomiasis being treated (WHO 2018). Other control strategies include provision of safe water, improved sanitation, hygiene education, and snail control.

1.6: Schistosomiasis control in sub-Saharan Africa

Control of schistosomiasis is based primarily on large scale treatment of population groups at risk with the antischistosomal drug praziquantel (PZQ). The WHO strategy for schistosomiasis control focuses on reducing disease burden through periodic, targeted treatment with PZQ (WHO, 2013). In order to reduce morbidity and limit transmission, considerable strides have been taken to control schistosomiasis in Africa (Fenwick *et al*., 2009). Control has been based almost entirely on the treatment of school children with PZQ, the only drug currently readily available for use. In very few cases have any attempts been made to control snails and associated production of cercariae. According to Gray *et al*. (2010), two features consistently emerge from such control efforts. One is that prevalence is significantly reduced, but never brought down to zero. Two, if control is relaxed, then rapid re-infections usually occur. This is not surprising because infected snails are present in local transmission sites and provide a persistent source of cercariae for new infections or re-infections. Also, parasite eggs continue to be released into snail habitats since not all the

infected people are treated, nor are the reservoir hosts (Hanelt *et al*., 2010). Nevertheless, drug-based control efforts reduce prevalence of infection and morbidity, may reduce the level of transmission and ultimately, reduce the genetic diversity of the parasite (Norton *et al*., 2010). However, they do not stop transmission which can quickly rebound. Consequently, schistosomiasis control depends on a continual application of PZQ which is by no means assured in most endemic countries of sub-Saharan Africa (Hotez *et al*., 2010). There is a growing consensus that the only hope for approaching the WHO's ambitious goal of schistosomiasis elimination by 2025 is to implement integrated control programs that include chemotherapy with PZQ as the backbone, supported by improved sanitation and hygiene, provision of safe water supplies, hopefully, a vaccine, and snail control (Rollinson *et al*., 2013; Mo *et al*., 2014; Secor 2014).

1.7 How schistosome-snail studies can improve control of schistosomes

First, by defining the level of compatibility between local snail and schistosome populations, we gain insights as to the extent to which control programs must lower egg input into snail habitats to be successful. For example, if compatibility is shown to be high and the number of infected snails is large, then prospects for achieving a meaningful level of control based purely on drug treatment dim considerably (Sturrock *et al*., 1994; Kloos *et al*., 1997). Second, if particular snail factors that influence compatibility are identified, they could be targeted for manipulation to diminish compatibility, just as mosquitoes can be manipulated to control malaria or arbovirus transmission (DeBarro *et al*., 2011; Meredith *et al*., 2011; Scolari *et al*., 2011). Other avenues of snail control also exist and remain under explored. One approach borrows from the "dilution hypothesis" which notes that the level of transmission of a particular disease can be diminished if it exists in a complex environmental milieu, where natural biotic interactions can diminish the force of infection (Johnson

& Thieltges 2010). With respect to *S. mansoni* in sub-Saharan Africa, many natural enemies of snails or schistosomes primarily other non-schistosome larval trematodes, occur in environments such as the Asao stream in Nyakach, Kisumu (Loker *et al*., 1993). Of particular interest will be the proportion of each *Biomphalaria* species collected that will be found to be positive for *S. mansoni* and the extent to which snails are infected with other potentially competing/predatory species of other trematodes.

CHAPTER TWO: LITERATURE REVIEW

2.1 Snail-related *S. mansoni* epidemiology

Once a *S. mansoni* miracidium has emerged from an egg and penetrated a snail, after about 4 weeks of larval development, daily shedding of cercariae begins. Cercariae shedding can persist for weeks and in some cases much longer for even more than a year under laboratory conditions (Mutuku *et al*., 2014), though this key variable remains poorly unknown for wild snails. Mortality of infected snails is an important factor in regulating schistosome transmission (Anderson & May 1979). The freshwater pulmonate snail, *Biomphalaria pfeifferi* is widely distributed in sub-Saharan Africa, and in Kenya, its distribution includes the tributaries feeding Lake Victoria, canals in major irrigation schemes on the Kano plains (in western Kenya) or in the Mwea irrigation scheme in central Kenya or other irrigation schemes in the country, and in small impoundments, and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along the coast of Kenya, while *B. sudanica* is mainly found in shores of lake Victoria but also occurs in Lake Jipe, Taveta in southern Kenya (Loker *et al.*, 1993; Brown 2005). Nonetheless, in Kenya populations of *B.pfeifferi* and of the schistosome it transmits are widely separated by regions of aridity (Loker *et al*.,1993), as a consequence, it is possible that *S. mansoni* exhibits a greater degree of compatibility with its local snail populations than it does with other populations of the same species further removed geographically.

Mortality of snails exposed to *S. mansoni* is another key factor in transmission of schistosomiasis. With respect to the issue of local adaptation, it has been suggested that snails exposed to *S. mansoni* from a sympatric source should have a higher survival rate than when the snails are exposed to an allopatric source of *S. mansoni* (Charbonnel *et al*., 2005). This is because selection often favors life history traits that allow the parasite to take maximum advantage of its host and allow the host to

grow, reproduce and survive with the parasite so that it will allow the next generation of the parasite with the next generation of snails which are compatible (Charbonnel *et al*., 2005). It is important from the standpoint of transmission to understand the factors dictating the duration of time that an infected snail can survive and persist in producing cercariae. Of particular interest with respect to survivorship of wild snails exposed to wild *S. mansoni* is how heterogeneous the survival rates of the exposed snails are. For example, do many of the exposed snails die before they reach patency and thus resemble female mosquitoes that often perish before they can transmit malaria? Do some snails show an extraordinary ability to withstand the effects of infection and survive considerably longer than other infected snails, or perhaps even longer than unexposed control snails? Such snails could potentially have a disproportionate effect on maintaining transmission, especially in areas where the human population is being regularly treated to eliminate adult worms.

Most infected *B. pfeifferi* collected in Kenya harbor a single *S. mansoni* genotype but a few harbor multiple genotypes, and the underlying factors that dictate this pattern is again unknown (Steinauer *et al*., 2008). It may indicate that most infected snails have encountered only a single parasite, or that multiple parasite genotypes might be routinely encountered but limited by snail defense response or competitive interactions among the parasites.

2.2: Parasite-host interactions and local adaptation

Theory predicts that a parasite should be more adapted to sympatric hosts than to allopatric hosts, and that the superior adaptation of a parasite to local hosts should be more pronounced when the hosts have discontinuous sporadic distribution rather than continuous distribution (Loker 1993; Ebert 1994; Thompson 1994; Morand *et al.*, 1996). Adaptation of parasites to their local hosts is a common phenomenon but not universal, and sometimes the pattern is even reversed (Kaltz &

Shykoff 1998). A number of factors including high rates of local extinction (such that co-evolutionary associations do not have a chance to develop), high rates of migration of host or parasite populations, or time lags in response may also break down or obscure patterns of local adaptation (Thompson 1994; Lively and Dybdahl 2000). Also because snail habitats (in this case streams) are subject to change depending on weather conditions, there might be flooding which could sweep out the entire snail colony or prolonged drought in which the snail population dies out, and be re-colonized by a genetically different pool than was previously present, such events have limited possibility for co-evolutionary interactions generating local adaptation (Kaltz and Shykoff 1998). In Kenya, as assessed by microsatellite analysis, *S. mansoni* from Mwea (central Kenya) and Kisumu (western Kenya) is genetically diverse (Agola *et al*., 2006; Agola *et al*., 2009). In addition to being genetically diverse, *S. mansoni* exhibits a relatively rapid rate of migration owing to existence of long-lived adult worms in mobile human hosts. By comparison, *B. pfeifferi* is a strong self-fertilizer (Charbonnel *et al*., 2005) and its movement is relatively limited owing to its restriction to aquatic habitats. Based on these considerations it might be expected that *S. mansoni* exhibits strong local adaptation to *B. pfeifferi*. This has not been addressed adequately in Africa with a reciprocal cross infection design approach using field-derived snails and parasites not subjected to the biases resulting from prior laboratory propagation, an approach that better represents the conditions in natural transmission sites.

2.3: *Biomphalaria spp* snails-*S. mansoni* susceptibility

All three taxa of *Biomphalaria* that occur in Kenya are susceptible to *S. mansoni* and contribute to transmission (Magendantz 1972; Frandsen 1979; Steinauer *et al*., 2008, 2009; Adriko *et al*., 2013; Mutuku *et al*., 2014; Lu *et al*., 2016). However, because of inherent differences in their biology,

their compatibility with *S. mansoni* could vary and so does their contribution to transmission and how it could be interrupted. It is expected that the strong preferential self-fertilizing species *B. pfeifferi* (Jarne and Theron 2001; Charbonnel *et al*., 2005; Campbell *et al*., 2010) will on average be significantly more susceptible to *S. mansoni* infection than either *B. sudanica* or *B. choanomphala*, both of which are preferential out crossers (Standley *et al*., 2014). This is because selfing does not afford the opportunity for genetic recombination considered to be strongly advantageous with respect to defense from parasites (King *et al*., 2011; Koskella *et al*., 2011; Singh *et al*., 2015). It is hypothesized that *B. sudanica* will be the least compatible of the three snail taxa, because it regularly experiences high levels of exposure to *S. mansoni* as well as to several other digenean species in its shoreline habitats (Rowel *et al*., 2015). The only digenean species known to commonly infect *B. choanomphala* in deeper waters is *S. mansoni*. The overall compatibility of *B. choanomphala* with *S. mansoni* is predicted to be on average intermediate between the other two *Biomphalaria* species (Frandsen 1979; Lu *et al*., 2016).

Previous compatibility experiments between *S. mansoni* and its snail intermediate hosts (the *Biomphalaria spp*) done in different studies have reported mixed results, with some snails being readily susceptible to both allopatric and sympatric *S .mansoni* isolates while others, exhibited reduced susceptibility to allopatric *S. mansoni* strain compared to sympatric isolates, with others returning high infection rates when exposed to allopatric *S. mansoni* than to sympatric *S. mansoni*. In Guadeloupe, a study by Prugnolle *et al*., (2006) that employed reciprocal cross infection experiment using 5 *B. glabrata* and 5 *S. mansoni* isolates from localities separate from each other by 2-10 kilometers reported relatively high overall mean susceptibility amongst all the combinations (52-78%). In Benin, West Africa, a study by Ibikounlé *et al.,* (2012) reported varying susceptibility levels for different snail strains after exposure to single *S. mansoni* miracidium per

snail. The study utilized an isolate of *S. mansoni* from Benin, 1 sympatric and 2 allopatric *B. pfeifferi* snail strains from Benin and 2 allopatric *B. pfeifferi* strains from Oman, in the Arabian Peninsula. The study established that the Benin sympatric combination and allopatric combinations, 1 allopatric combination with Oman snails had high infection rates (>80%), however 1 allopatric combination with Oman snails had significantly low infection rate (46.43%). Another study by Adriko *et al*., (2013) demonstrated that all Ugandan *Biomphalaria spp* snails including *B. sudanica* and *B. choanomphala* from different locations were equally susceptible to the *S. mansoni* isolates from sympatric or allopatric origin. Their study suggested that when the parasite isolate from a different geographical origin is used for infection, survival of infected snails increased, leading to an increased transmission potential. Susceptibility of snails to the parasite is very key in determining the force of transmission of the parasite from intermediate host to humans who are the definitive hosts. The more susceptible the snails are, the more snails that become infected and the more cercariae that will be produced which increases chances of more people becoming infected.

2.4: Amphistomes and echinostomes as potential enemies and biocontrol agents against schistosomes.

There is a growing appreciation for the role of ecological complexity with its attendant checks and balances in limiting the transmission and potential for emergence of infectious diseases (Johnson and Thieltges 2010). Schistosomiasis is no exception, in addition to hosting *S. mansoni* (Loker *et al*., 1981), *Biomphalaria* snails support the larval development of at least 31 other trematode species in tropical Africa. Larval digenetic trematodes engage in strong intra-snail competitive and/or predatory interactions with one another, and schistosome larvae are frequently (but not always) eliminated as a consequence. The Asao stream in Nyakach, Kisumu and the Tilapia beach

and many other beaches within the shores of Lake Victoria are typical of any schistosome transmission site in sub-Saharan Africa, as they support many other vertebrate species including domestic ruminants, fish, frogs, and birds, all potentially capable of harboring trematode species dependent on *Biomphalaria* snails for their transmission. Preliminary snail sampling in Asao stream and the Tilapia beach has established the presence of *S. mansoni* and other trematodes (amphistomes, strigeids, xiphidiocercariae and echinostomes) cycling through *Biomphalaria* snails (Martina *et al*., 2016). Amphistome flukes (Superfamily Paramphistomoidea), are ubiquitous in sub-Saharan Africa (Brown 2005), and some evidence suggests that amphistome larvae can facilitate the development of schistosome infections in some snail species (Southgate *et al*., 1989), but it is likely that if present in a snail they will pose a major barrier to schistosome colonization and development (Hechinger *et al*., 2011). The direct predatory and/or competitive impacts of amphistomes and echinostomes rediae have on *S. mansoni* sporocysts are not known, nor is any information available regarding their indirect effects on influencing compatibility of snails to *S. mansoni* or in causing premature snail mortality. It is expected that by virtue of being stressed by their prior infections, the snails will die before *S. mansoni* can complete its development, or that the pre-existing larvae of other trematodes within will prevent *S. mansoni* infections from succeeding and thus be unable to colonize snails infected with amphistomes or echinostomes prior to, or at the same time it is receiving *S. mansoni* infections. All of these possibilities are of interest and clearly will have an impact on *S. mansoni* transmission.

2.5: Schistosomiasis transmission and snail - *S. mansoni* compatibility

Human schistosomiasis is transmitted throughout the sub-Saharan Africa, and cercariae, the larval schistosomes that develop in the snail, are the human infective stages, these are shed by infected

snails into water where they swim freely to find the definitive host (Rollinson 1987). They penetrate intact skin of the definitive host, and enter into the circulatory system and migrate to the liver and mesenteric system, where they develop into adult worms. A key determinant of transmission success is compatibility of the local snails with schistosome parasites. Compatibility here is defined as the likelihood that exposure of a snail to a miracidium or miracidia leads to a cercariae-producing infection. The greater the compatibility, the more infections that result from a given level of schistosome egg input into the habitat, the more cercariae that are produced, and the harder it is for drug-based control to have a lasting effect on reducing transmission. This is one parameter that is rarely explicitly incorporated into models of schistosomiasis transmission (French *et al*., 2010) because of limited information on true level of compatibility between natural African snails and schistosome populations.

In Guadeloupe, Theron *et al.*, (2008) revealed that because both *B. glabrata* and *S. mansoni* populations are genetically diverse, single miracidium infections result in low infection rates (about 10%). Based on their matching allele's model, similar considerations likely apply in sub-Saharan Africa. Snails and especially schistosomes very quickly lose genetic diversity when reared in the laboratory (Jones-Nelson *et al*., 2011), nullifying any chance of studies of laboratory strains to reveal natural compatibility levels. Also, as with other parasites, it is expected that if particular circumstances are met, schistosomes will become more adapted to snails within the local populations that transmit them as opposed to snails from more distant areas (Prugnolle *et al*., 2006). Knowledge on the magnitude of such "local adaptation" for field-derived schistosomes and snails in Africa remains limited.

Regarding the compatibility between *S. mansoni* and *Biomphalaria* snails, few studies have assessed compatibility using genetically diverse specimens taken from the field in Africa. One

laboratory study using recent field isolates from Senegal showed a high infection level (50%) for snails exposed to one miracidium per snail (Southgate *et al*., 2000). In this case, ecological changes had favored the rapid spread of *B. pfeifferi* snails that were remarkably uniform genetically (Campbell *et al*., 2010), and that had little resistance to schistosome infection. The concept of low snail-schistosome compatibility rates in endemic foci is reinforced by PCR-based surveys of *Bulinus nasutus* snails from natural populations (Hamburger *et al*., 2004). These studies indicated that snails often harbor much higher numbers of pre-patent than patent (cercariae-producing) *Schistosoma haematobium* infections, suggesting that low compatibility limits the ability of snails to produce cercariae (Hamburger *et al*., 2004) thus slowing down transmission of the parasite from the intermediate host to humans. Limited data is available for field-derived *Biomphalaria* species in Africa.

2.6: Problem statement

Schistosomiasis control strategies in Africa rely almost exclusively on treatment of the infected individuals with the anti-schistosomal drug, praziquantel (PZQ), and although this rapidly reduces infection prevalence and can lessen the intensity of infection, it is incapable on its own of interrupting transmission (King *et al*., 2006; Lelo *et al*., 2014; Secor 2014; Ross *et al*., 2015). Where transmission intensity is high, re-infections to the human population is likely to be more frequent and therefore, the harder it will be for chemotherapy based-control strategies to have a long lasting effect. Preventive chemotherapy has not been sustainable partly because it doesn't interfere with snails in the transmission sites and its associated production of cercariae. A better undertanding of the magnitude of compatibility between field derived schistosomes and their natural snail intermediate hosts in Africa remains largely unknown. This might lead to the

identification of snail factors that influence compatibility, which can then be manipulated to diminish compatibility thus, reduce transmission and compliment chemotherapy-based control strategies.

2.7: Expected application of results

Findings from the present study are of great relevance to understanding the factors that influence *S. mansoni* transmission in nature that could be exploited for schistosomiasis control and elimination. In particular, the study provides an opportunity to obtain real estimates of compatibility of the 3 *Biomphalaria* snail species commonly present in Kenya, with *S. mansoni* and further provides insights on how the distribution of the 3 species of *Biomphalaria* snails found in various localities in the country could be influencing the epidemiology of intestinal schistosomiaisis in Kenya. The potential impact of amphistomes and echinostomes rediae on *S. mansoni* sporocysts development, and in diminishing the force of schistosome transmission to people is useful information that can be utilized in the formulation of novel integrated control strategies towards achieving the WHO goal of eliminating schistosomiasis by 2025.

2.8: Research questions

i. Does snail size, species or snail collection locality influence compatibility with *S. masoni?*
ii. Does vectorial competence of *B. choanomphala* differ from that of *B. sudanica* as intermidiate host snails of *S. mansoni* in Lake Victoria?

iii. Does snail - schistosome compatibility contribute to locality differences in outcomes of chemotherapeutic intervention to control and interrupt transmission in western Kenya?

2.9: Null hypotheses

i. Compatibility between *B. sudanica* and *B. pfeifferi* cross infected with *S. mansoni* transmitted by either of the two intermediate host snails is not different.

ii. Vectorial competence of *B. choanomphala* is not different from that of *B. sudanica* as intermidiate host snails responsible for transmission of *S. mansoni* in Lake Victoria?

iii. *Biomphalaria* snail's species and population composition is not a determining factor in differences observed in outcomes of chemotherapeutic intervention among different villages in western Kenya.

2.10: Objectives

2.10.1: General objectives

To determine compatibility of *B. pfeifferi, B. sudanica and B. choanomphala*, commonly present in Kenya, with local populations of *S. mansoni* and determine if snail-*S. mansoni* compatibility is a factor in the differences in infection response to PZQ treatment observed in some localities in western Kenya.

2.10.2: Specific objectives

i. To determine infection success rates and longevity of wild *B. pfeifferi* and *B. sudanica* after exposure to sympatric or allopatric *S. mansoni*, and how it is influenced by snail size, species or collection locality.

ii. To determine vectorial competence of *B. choanomphala* and *B. sudanica*; snail intermidiate host of *S. mansoni* in Lake Victoria, Western Kenya.

iii. To determine if *Biomphalaria* snail's species and population composition is a determining factor in locality differences observed in outcomes of chemotherapeutic intervention among different villages in western Kenya.

CHAPTER THREE: GENERAL MATERIALS AND METHODS

3.1: Study area.

Snails for laboratory-based experiments were collected from localities within 3 counties in Kenya; 1) Asao stream, Jimo east village, Nyakach sub county and Nawa beach, Kisumu central sub county, Kisumu county; 2) Anyanga beach, Kanyibok village, Bondo sub county, Siaya county; 3) Mukou stream, Mukou village, Mwea sub county kirinyaga county. Stool samples for isolation of schistosome eggs were also collected from school children or adults in villages were the snails were collected. These sites were selected because prevalence of schistosomiasis is high (> 50%) and are easily accessible by road. For field-based experiments in Aim 3, sampling habitats were located in 10 villages in the shores of Lake Victoria in Kisumu and Siaya counties, these sites had been identified and defined in a previous study (Wiegend *et al*., 2017).

3.2: Study design

This was both a field and laboratory based experimental study which had 3 main arms

1) Reciprocal cross infection experiments to determine compatibility between *S. mansoni* and its 2 most important local intermediate host snails (*B. pfeifferi* and *B. sudanica*) in Kenya in relation to snail's size and age.
2) Reciprocal cross infection experiments to determine competence of *B. choanomphala* and *B. sudanica*; intermediate host snails of *S. mansoni* in Lake Victoria, Western Kenya.
3) Field sampling of transmission sites determine if *Biomphalaria* snail's species and population composition is a determining factor in locality differences observed in outcomes of chemotherapeutic intervention among different villages in western Kenya.

3.2.1: Determination of compatibility between *S. mansoni* and its 2 most important local intermediate host snails (*B. pfeifferi* and *B. sudanica*) in Kenya in relation to snail's size and age

In this study, reciprocal cross infection experiment was conducted between *B. pfeifferi* from Mwea (Kirinyaga county) and *B. sudanica* from Nawa (Kisumu county) with *S. mansoni* from either Mwea or Nawa. Snails for exposure in this experiment were collected from Nawa beach (Kisumu county) and Mukou stream (Kirinyaga county) and screened for any form of infection. Only snails not shedding any form of cercariae were used. The snails were allowed to adopt in the out-door rearing facility for 1 week before the experiments were set up. After lapse of the 1 week, the snails were screened again for any infections before the experiments were set up. Some snails may harbor pre-patent infections and to control for this, some of the snails were left unexposed in the experimental design to serve as a control. Non-shedding snails were divided into 3 groups (juveniles <6mm shell diameter, young adults 6-9mm, and older adults >9mm). Based on other previous similar studies (Ibikounlé *et al*., 2012; Southgate *et al*., 2000; Prugnolle *et al*., 2006)100 snails in each category were individually exposed to 1 miracidium each from either Nawa or Mwea, or left unexposed as illustrated in figure 3.1. The dose of one miracidium/snail is important because it provides the best estimate of compatibility: one parasite genotype versus one host genotype. The snails were held in the out-door snail facility and screened for infection starting 3 weeks post-exposure (PE) for 6 months, any snail that sheds cercariae at 3 weeks PE were assumed to be having a pre-patent field infection and were excluded from the experiment. At 6 weeks PE the number of cercariae that came out of each individual shedding snail were enumerated and this was repeated once every month. A total of 1800 snails and 1200 *miracidia* were used in this experiment.

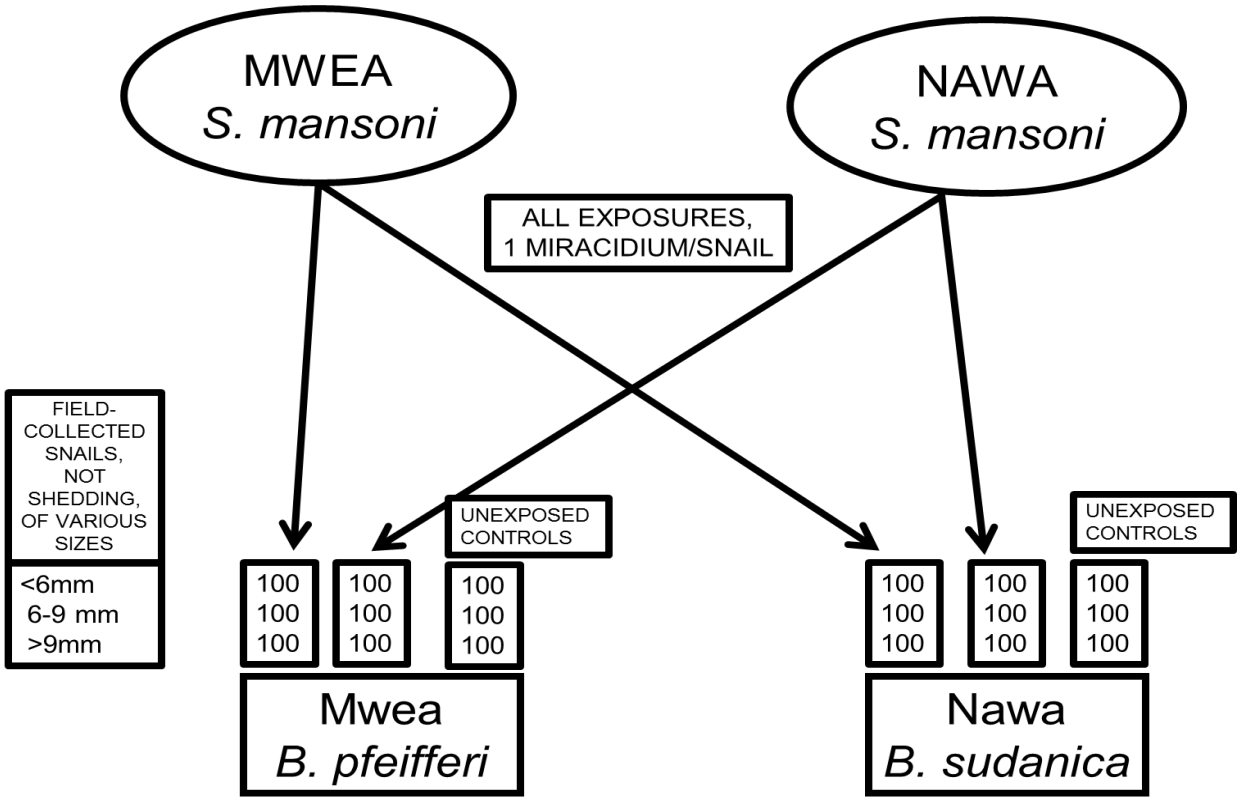


Figure 3.1: Diagramatical presentation of reciprocal cross-infection of *B. sudanica* from Nawa and *B. pfeifferi* from Mwea with *S. mansoni* from either of the two locations**.**

3.2.2: Comparative competence of *B. choanomphala* and *B. sudanica*; intermediate host snails of *S. mansoni* in Lake Victoria, Western Kenya.

In this experiment, reciprocal cross infection experiment was conducted using laboratory raised F1 generation *B. choanomphala* and *B. sudanica* both from Anayanga beach, Kanyibok, Siaya county and *B. pfeifferi* from Asao stream, Nyakach, Kisumu county. Use of F1 generation snails was necessary because of the size diffrences between the two snail species hence this ensured that snails used in this experiment were of the same age. For each of the 2 snail species, 3 groups each of 100 snails were exposed to either 1, 5 or 10 sympatric (derived from the same location as the snails) *S. mansoni* miracidia and another similar set was exposed either 1, 5 or 10 allopatric (derived from a different location as the snails) *S. mansoni* miracidia. For each of the 2 species, a group of 100

snails was set up but not exposed to any miracidia and served as control as illustrated in figure 3.2. The snails were held in the out-door snail facility and screened for infection starting 4 weeks PE for 3 months. At 6 weeks PE the number of cercariae that came out of each individual shedding snail were enumerated.

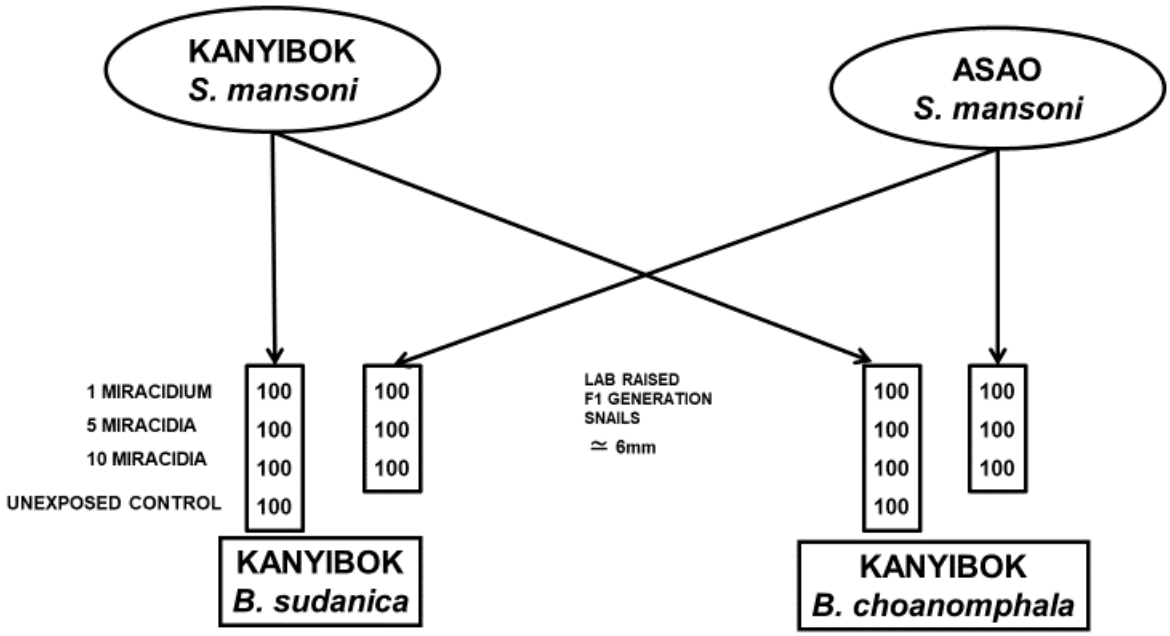


Figure 3.2: Diagrammatical presentation of reciprocal cross exposure experiment of *B. choanomphala* and *B. sudanica* using either allopatric or sympatric *S. mansoni*

3.2.3: Determining if *Biomphalaria* snail's species and population composition is a determining factor in locality differences observed in outcomes of chemotherapeutic intervention among different villages in western Kenya by use of seasonal snails' survey and sentinel mice.

In this experiment, the force of transmission was assessed in 10 villages (6 villages where there was persistent high prevalence rate of *S. mansoni* in school children and 4 responsive villages were

there was significant reduction in prevalence rates of *S. mansoni* in school children after 4 consecutive annual MDA with praziquantel). In each of the villages, two possible transmission habitats were identified based on presence of *Biomphalaria* snails and human water contact, in which transmission was assessed using 5 floater cages with 2 mice in each. This was done 4 times so as to capture data during different seasons in a year. Swiss albino mice weighing 18-20 grams were used in this experiment, the mice were exposed to the lake water at mid-day for three hours (11:00 - 14.00 hrs). Swiss albino was used because they are susceptible to *S. mansoni*, are docile and easy to handle and are easy to maintain in a laboratory. The mice were then returned to the laboratory in KEMRI Nairobi were they were maintained at animal care unit for 8 weeks PE to allow any infections to mature after which they were perfused to recover the mature worms. A total of 800 mice were used (10 villages X 2 habitats per village X 5 cages per habitat X 2 mice per cage X 4 sampling times)

Immediately following exposure of the mice as described above, snails survey for *S. mansoni* and other digenetic trematode infection was done by two methods: **1) Scooping** from the lake shore by two individuals for 15 minutes using long-handled scoops (stainless steel sieve with a mesh size of 2 × 2 mm, supported on an iron frame) in the same habitat in which mice were exposed. **2) Dredging** off shore adjacent to the habitats were mice exposure was done as described by Magendantz (1972) using a standard dredge with a nylon rope tied in the handle and tethered into a boat, the dredge was drawn in continuous stretches 150 meters off shore so that it scours the bottom of the lake. The dredged materials were put in a wide basin and then washed off in a sieve to check for presence of any snails. Snails of all species collected were returned to the laboratory at KEMRI, Kisian, were they were sorted into species level, cleaned, and isolated into individual wells in plastic 24 well culture plates with 1 ml of dechlorinated tap water and allowed to stay

overnight to check for shedding of schistosomes or other trematodes infections. Snails not shedding any cercariae were maintained in the semi field snail structure for one month post collection time and screened again to determine if they were harboring any pre-patent infection.

3.3: Field collection and maintenance of snails

Snails were collected at random from various snail habitats using a standard scoop by the method described by Coulibaly and Madsen (1990). The scoops were made from stainless steel sieve with a mesh size of 2 × 2 mm, supported on an iron frame mounted on a 1.5 meter long wooden handle. Snails were then sorted out and identified to species level based on shell morphological characteristics using standard taxonomic keys as described by Brown (2005) and DBL-WHO (1998). The snails were held in an out of doors, "semi-field" ambient conditions in a simple, roofed, open-sided structure containing benches (figure 3.3) on which plastic snail aquaria were maintained under the shade of the roof in KEMRI grounds at Center for Global Health Research (CGHR), Kisian, Kisumu. The goal was to promote more natural survivorship rates of exposed snails by having environmental conditions much closer to what snail's experience in the field where they thrive well. Aquaria were filled with aged dechlorinated tap water which was changed once a week and aerated by connecting a 4 millimeters thick pipe to an air pump to keep it fresh. Sterile crushed oyster shells were provided as substrate and the snails were fed on slightly boiled lettuce.

Figure 3.3: An "outdoor" snail rearing facility in KEMRI, Kisumu campus

3.4: Collection, isolation and hatching of *S. mansoni,* amphistosome or echinostome ova to miracidia

School children aged between 6-12 years from Mukou, Kanyibok and Obuon primary schools and fisher men from Nawa village were screened for *S. mansoni* infection. This was done by collecting individual fecal samples then prepared Kato Katz thick fecal smear as described by Katz *et al.* (1972). Briefly, the fecal sample was mixed using a wooden tongue depressor, a small quantity was then taken and strained through a stainless steel sieve with 200 micrometer pores. A plastic template was placed on top of glass microscope slide and its aperture filled with the sieved feces. Carefully the template was removed and a cellophane strip soaked in malachite green stain for at

least 24 hours placed over the glass slide to cover the fecal sample. The slide was then inverted into a blotting paper and gently, even pressure was applied to spread the fecal sample to a disc about 3/4 inch in diameter. The slide was then left to dry and clear for 24 hours before checking for presence of ova using a compound microscope. 5 children from each of the schools, 5 sand harvesters or fishermen with the highest egg intensity were recruited in this study. Fecal samples were collected from each of the 5 individuals and then pooled together according to the site of origin. Eggs were isolated by homogenizing the fecal sample with dechlorinated tap water using a blender, followed by filtering through a series of nested sieves of different pore sizes in descending order (710 µM, 425 µM, 212 µM, and 45 µM). The filtrate retained by the 45 µM sieve which contains the *S. mansoni* eggs was then washed off and put in a glass conical flask that contained dechlorinated tap water and placed under indirect sunlight to allow hatching. For ease of collecting miracidia, the flask was covered with black polythene paper to the neck level to allow collection of the positively phototrophic miracidia in the flask's neck. Using a plastic pipette, the top most water from the flask was transferred to a glass petri dish and with the aid of a dissecting microscope, the miracidia were collected using a micropipette and transferred into 24 well plastic culture plates. Snails of an appropriate size were then introduced into the miracidia in the culture plates, one snail in each well (figure 3.4) and then left in indirect sunlight for 6 hours for the miracidia to penetrate into the snails' body tissue before being transferred back to the aquaria.

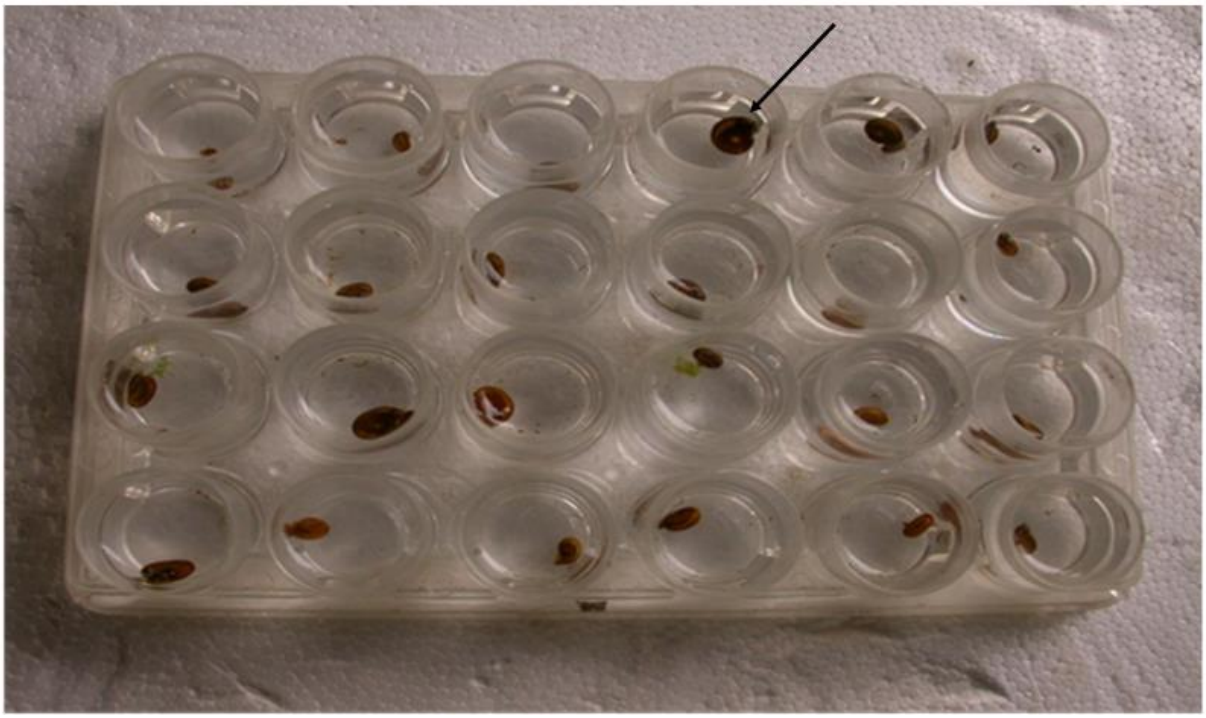


Figure 3.4: Snails being exposed to miracidia in a plastic 24 well culture plate

3.5: Determination of snail's mortality and screening of snails for infection.

Snail's survival and mortality were recorded on weekly basis, snails that did not respond to food items (slightly boiled lettuce), or move and remained retracted in the shell were considered dead. To determine infection rate among the snails, shedding was done on a weekly basis from the third week after exposure by screening the snails from 10.00 hrs to 12.00 hrs which is the pick time for shedding of *S. mansoni* cercariae. Each individual snail was put in a 1ml volume of dechlorinated tap water in 24-well plastic culture plates and placed in an indirect sunlight. Individual wells were then examined under a dissecting microscope for presence of cercariae. If enumeration of number of cercariae produced by each individual infected snail was required, two aliquots of 50 µL each were picked using a micropipette from each of the well and placed separately in a gridded petri dish. Two drops of Lugol's iodine were then added to stain and immobilize the cercariae. They

were then counted under a dissecting microscope with aid of a tally counter and the average from the two aliquots calculated and then multiplied by 20 to get the total number of cercariae that were produced by the specific snail for the 2 hours. This procedure was followed for all the shedding snails.

3.6: Exposure of sentinel mice to water in transmission habitats

Exposure of sentinel mice was done as described by Chandiwa (1987). Briefly, for ease of penetration of cercariae into the mice, the belly region was shaved using an electric shaver and the mice were put into floater cages. The floating cages were made from plastic containers measuring 20 X 14 X 7 cm, the bottom of the container was cut off and a wire mesh with apertures measuring 5 x 5 mm fixed using adhesive tape and glue. The lid of the container was perforated with circular apertures of 5 mm diameter to allow enough circulation of air. To ensure the containers do not sink in the water, styrofoam blocks were attached in the sides of the container so that only 1 cm of the floater is submerged into the water, this set up allowed the mice belly region to be submerged approximately 5 mm into water (figure 3.5). A nylon string measuring approximately 4 meters was attached into the floater and used to tether the floater in surrounding vegetation so that it doesn't get swept away by water currents.

Figure 3.5: Floating mice cage used for exposing sentinel mice in to water in a human water contact point in the shores of lake Victoria

3.7: Maintenance of experimental mice

Mice were maintained at animal care unit in KEMRI, Nairobi under a 12:12 hour light-dark cycle at 25ºC. They were kept in plastic cages measuring 30cm X 15cm X 15cm with a metallic lid and fed on commercial mice pellets and provided with clean tap water *ad libitum*. Dry wood shavings were used as beddings, this were changed after every 3 days. Each of the cage housed 5 mice.

3.8: Mice perfusion (recovery of worms from experimental mice)

Perfusion was done 8 weeks post-exposure as described by Tamarath *et al.*, 1947. Briefly mice were euthanized by intra-peritoneal injection of sodium pentobarbitone (sagatal) and their death

was verified by absence of heart beat and respiration for one minute, blanching of the eye ball and lack of response to a foot pinch. This procedure is consistent with the recommendations of the panel on euthanasia of the American Veterinary Medical Association, and is acceptable to KEMRI's animal care and use committee. Using scissors and forceps, the peritoneal cavity was opened up and the rib cage and diaphragm cut off to expose the heart, care was taken to ensure the circulatory system remains intact. The mouse was then clamped into the dissection apparatus and a cut made in the portal-hepatic vein. To flush out the worms from circulatory system, 50 milliliters of perfusion fluid were then pumped into the circulatory system using a syringe and needle through an insertion in the left ventricle of the heart. Recovered worms were enumerated and their sex determined by morphological features, they were then preserved in absolute ethanol and stored at -20^{o}C. The mice carcasses were then disposed by incineration.

3.9: Study approvals and ethical considerations

Ethical and scientific approval for this study was obtained from the KEMRI Scientific and Ethics Review Unit (SERU), referenced SSC No. 2373 and SSC No. 1820 and from University of New Mexico (UNM) Institutional Review Board and referenced: 18115. Children were selected for this study because they are the most vulnerable to schistosomiasis, contribute significantly to environment contamination and parasite transmission, and are easily accessible from their schools. Likewise, sand harvestrers and fishermen are vulnerable because of nature of their activities they do and are easily accessible from the beach sites. Prior to recruitment, screening, selection, or treatment of any children, the purpose of the study was explained to the school administration and children's parents/guardians in a language understandable by the local residents. Also to the fishermen, the purpose of the study was explained to them in a language they easily understood.

Participation was voluntary and participants were allowed to withdraw at any time, without penalty. Written and signed consent were sought from the fishermen and parents/guardians of the children. Involvement of human subjects in this study was limited to collecting fecal samples which is a non-invasive procedure, with no perceived risks, and is routinely used in clinical investigations of intestinal infections. Any participant found positive for *S. mansoni* was offered standard treatment with praziquantel (40mg/kg body weight) and those found positive for geohelminths (*Ascaris*, hookworms, *Trichuris*) were offered treatment with albendazole (500 mg) by a trained and qualified clinician. Praziquantel and albendazole are remarkably safe drugs and have been administered to millions of people in endemic countries, with no or only transient side effects. To ensure confidentiality, each participant was given a personal identification number as an identifier, and all future references to information/data obtained from the participant were referred to by this number. Use of mice in this study was approved by KEMRI animal care and use committee (ACUC) permit number KEMRI/ACUC/03.10.15. All protocols and practices in this study for the handling and manipulation of mice were in accordance with the guidelines of the American Veterinary Medical Association (AVMA) for humane treatment of laboratory animals. Authorization to undertake this research was obtained from National Commission for Science, Technology and Innovation (NACOSTI) Permit number NACOSTI/P/16/9609/12754, National Environment Management Authority (NEMA) Permit number NEMA/AGR/46/2014 and Kenya Wildlife Services (KWS) permit number KWS/BRM/5001.

3.10: Statistical data analysis

Data analysis was conducted using Graph pad Prism version 7 statistical and graphing software and IBM SPSS version 21.0 statistical software and Microsoft Excel. Descriptive statistics such as

proportions were used to summarize categorical variables while measures of central tendency such as mean, standard error, and range were used to summarize continuous variables. Odds Ratio (OR) and 95% Confidence Interval (CI) were used to estimate the strength of association between outcome and exposure variables. To determine if PHS sites differed from RESP sites in snail-related factors, they were compared with respect to relative abundance of each snail (*B. sudanica* or *B. choanomphala*), the prevalence of infected snails, and the number of adult schistosome worms collected from the sentinel mice (as a measure of the force of infection). To determine effects of snail species and parasite source on infection status (yes/no) at the three different doses, a generalized linear model was used with a binomial distribution and logit link function due to the binary response variable. Thereafter, models were split by snail species in order to detect parasite source differences at three different doses within a species. For the latter individual species models, parasite source was nested within dose and dispersion was tested by a chi-squared test. Estimates and confidence intervals (CI) were back transformed to represent odd ratios. To determine whether snail species, parasite source and dose have an effect on the number of cercariae produced, a generalized linear model with a negative binomial distribution and a log link function were fitted to account for the response variable (number of cercariae) being count data and over dispersion.

CHAPTER FOUR: COMPARISON OF KENYAN *BIOMPHALARIA PFEIFFERI* AND *B. SUDANICA* AS VECTORS FOR *SCHISTOSOMA MANSONI,* AND THE EFFECTS OF SNAIL BREEDING SYSTEMS ON TRANSMISSION

4.1: Introduction

Vector-borne diseases including malaria, dengue, Zika virus, and trypanosomiasis continue to pose major challenges to public health (Smith *et al*., 1998; Greenwood *et al*., 2002; San Martín *et al*., 2010). Similarly, snail-transmitted infections also remain problematic in the developing world, with snails playing an indispensable role in transmission (WHO, 2016). With a few exceptions, digeneans (digenetic trematodes or flukes) use snails as first intermediate hosts, enjoying a remarkably productive period of asexual reproduction within snails that culminates with the production of cercariae that may continue for months and in some cases, over a year (Mutuku *et al*., 2014). The prolonged production and release of numerous cercariae into the environment gives the life cycles of human-infecting schistosomes considerable stability, thereby challenging control efforts. Given the vast populations of snails that occupy many natural transmission sites, control of snail-transmitted diseases is a formidable challenge. Schistosomiasis control has been most successfulwhen snail control has been implemented (Lelo *et al*., 2014; Njenga *et al*., 2014; Sokolow *et al*., 2016), highlighting the importance of knowing more about the biology of the snail hosts and their interactions with snail-transmitted parasites of human and veterinary concern.

The competence of snails to serve as hosts for schistosomes is influenced by several different factors. These include infection prevalence as measured by the proportion of schistosome-exposed snails that actually shed cercariae, length of time required to complete sporocyst development for the first release of cercariae following exposure to infection (the pre-patent period), survival period of infected snails, duration of actual shedding of the schistosome-exposed snails and daily output

of cercariae from infected snails (Ibikounlé *et al*., 2012). It is also important to appreciate that schistosome snail hosts exist in complex environmental settings that can influence their capacity to support transmission. They must simultaneously cope with exposure to potential infection with several other species of digenetic trematodes, which may even be more common than schistosomes (Loker *et al*., 1981; Mohammed *et al*., 2016), and that also have the potential to cause castration, thereby strongly affecting fitness of the snails. Moreover, infection with other trematode species may alter susceptibility to infection with schistosomes (Spatz *et al*., 2012). Finally, the suitability of snail environments often varies dramatically with season (Charbonnel *et al*., 2005) which is anticipated to influence the snail's breeding system (for instance, selfing vs. out-crossing) and which in turn might influence their competence in resisting infection by parasites (Howard *et al*., 1994; Gibson *et al*., 2016).

In Africa, transmission of *S. mansoni* is enabled by 12 species of *Biomphalaria* with *B. pfeifferi* and *B. sudanica* being the most prominent intermediate hosts in Kenya (Loker *et al*., 1993; Brown, 1994). *B. pfeifferi* is widely distributed in tributaries feeding Lake Victoria, canals in Mwea irrigation scheme in central Kenya, and in small impoundments, and in both seasonal and perennial streams throughout the country, except in the tropical lowland belt along the Kenyan coast. *B. sudanica* is mainly found along the shores of Lake Victoria and other larger water bodies like Lake Jipe (Loker *et al*., 1993; Brown, 1994).

Previously, a series of studies exploring the role of Kenyan *Biomphalaria* snails in the transmission of *S. mansoni* have been undertaken. Mutuku *et al*. (2014) examined the compatibility of field-derived *B. pfeifferi* to *S. mansoni* miracidia obtained from infected school children with a particular interest in learning if *S. mansoni* exhibits a greater degree of compatibility with its local *B. pfeifferi* snail populations than it does with other *B. pfeifferi* populations further removed geographically.

Both sympatric and allopatric combinations of parasites and snails exhibited high compatibility (approximately 50% at a dose of 1 miracidium per snail), with an increase in infection and mortality rates as the miracidial dose was increased. Approximately 3% of *B. pfeifferi* from Asao, western Kenya, exposed to a low dose of sympatric miracidia (1 or 5) continued to shed cercariae for as long as 58 weeks post exposure (PE). In another study by Lu *et al.* (2016) that compared *B. pfeifferi* and *B. sudanica* with respect to their role in transmission of *S. mansoni* by use of a PCR assay to detect *S. mansoni* in snails, it was established that during 24 days of pre-patent development following exposure to a single *S. mansoni* miracidium, 48.3% of *B. pfeifferi* harbored successfully developing parasites as compared to only 23.5% for *B. sudanica*. At 40 days PE, by which time it was expected that any successful infection should have culminated in cercariae production, only 14.7% of *B. sudanica* had either shed cercariae or harbored viable parasites, whereas the comparable figure for *B. pfeifferi* was 47.6% (Lu *et al*., 2016). These results are suggestive that *B. pfeifferi* offers a more conducive environment for schistosome development than *B. sudanica* during the pre-patent period.

In contrast to the study by Mutuku *et al*., (2014) which dealt exclusively with *B. pfeifferi*, the aim of this study was to examine the relative compatibility of field-derived Kenyan *B. pfeifferi* and *B. sudanica* to *S. mansoni* and determine if *B. pfeifferi* was more susceptible to infection with *S. mansoni* derived from a sympatric locality, one in which its transmission likely depended exclusively on *B. pfeifferi,* and if this snail species would be equally susceptible to *S. mansoni* taken from an allopatric area where *B. sudanica* routinely transmitted the parasite. Conversely, is *B. sudanica* more compatible with *S. mansoni* derived from the same location where it is routinely transmitted by *B. sudanica*?. This study also sought to document other parameters associated with

vectorial competence including length of pre-patent period, the number of cercariae produced daily, and the duration of shedding of cercariae by infected snails.

4.2: Materials and methods

4.2.1: Parasite and snail sources

S. mansoni eggs were obtained from pooled fecal samples from 5 school children aged 6-12 years from both Mukuo village, Mwea, Kirinyaga County, Central Kenya (GPS coordinates 00°40'54"S, 037°20'36"E, altitude 1,098 m) and Nawa village, Kisumu County, western Kenya (GPS coordinates 00°06'12"S, 034°42'75"E, altitude 1,272 m). Schistosome eggs were concentrated and hatched, and miracidia used to infect snails as described previously in chapter three. Snails were collected from the field from Mukou stream, in Mwea, central Kenya, and *B. sudanica* were collected from Nawa beach, on the shores of Lake Victoria, in Nawa village, Kisumu. Identifications were confirmed as *B. sudanica* or *B. pfeifferi* based on known geographical and habitat preferences (Loker *et al*., 1993; Brown, 1994; Dejong *et al*., 2001; Dejong *et al*., 2003; Steinauer *et al*., 2009) and conchological characters (Brown, 1994). The two species are distinct genetically and were not found coexisting in the habitats examined. *Biomphalaria* snails collected were isolated and screened for digenean infection and any snail found to be shedding cercariae of any type was discarded. Prior to exposure to *S. mansoni*, all non-shedders were maintained for an additional 4 weeks in an aquarium, both to adapt the snails to laboratory conditions and to permit re-screening to determine if they were still negative for digenean infections (Mutuku *et al*., 2014).

4.2.2: Experimental design

A reciprocal cross infection experiment was conducted whereby *B. pfeifferi* snails from Mwea and *B. sudanica* from Nawa were exposed to *S. mansoni* miracidia from either Mwea or Nawa. Snails

to be exposed to *S. mansoni* were categorized into 3 groups depending on size (shell diameter)/age: 1) juveniles < 6 mm shell diameter; 2) young adults 6–9 mm; 3) and adults > 9 mm (a total of 6 sympatric and 6 allopatric combinations). For each of the 12 possible combinations, 100 pre-screened snails found not to be shedding any digenetic trematodes, were exposed to 1 *S. mansoni* miracidium each. For each of the 3 snail size categories from the 2 locations, a group of 100 snails was not exposed to the parasite and served as unexposed controls. A total of 1,800 snails and 1,200 miracidia were used for this experiment. Starting at 1 week PE, the snails were examined once a week, for any snails shedding *S. mansoni* cercariae, for over a period of at least 24 weeks, or until the snails died using the procedure described below. Snails were counted, screened individually for evidence of shedding schistosome or any other cercariae, and the number of surviving snails recorded. For snails that were found to be shedding, the total number of cercariae produced for 2 hours between 10:00 hrs-12:00 hrs was determined as described in chapter 3.

4.2.3: Ethical statement

Approval for this study was obtained from the KEMRI Scientific and Ethics Review Unit (SERU) and was referenced SERU SSC No. 2373 and from the University of New Mexico (UNM) 3+9Institutional Review Board and referenced: 18115. Children were selected for enrollment into the study because they are the most vulnerable to schistosomiasis, contribute significantly to environment contamination and parasite transmission, are easily accessible from their schools, and are regularly offered treatment. Recruitment of human study subjects, their participation and care was done as described previously in chapter 3. Consent to participate in the study was obtained from parents or guardians. The information and data obtained from the study participants were stored securely within KEMRI on password-protected computers. This study was conducted with

the approvals of the National Commission for Science, Technology and Innovation (NACOSTI), Permit NACOSTI/P/16/9609/12754, and the National Environment Management Authority (NEMA), Permit NEMA/AGR/46/2014.

4.2.4: Statistical analyses

Data analysis was conducted using IBM SPSS version 21.0 statistical software and Microsoft Excel. Descriptive statistics such as proportions were used to summarize categorical variables while measures of central tendency such as mean, standard error, and range were used to summarize continuous variables. Odds Ratio (OR) and 95% Confidence Interval (CI) were used to estimate the strength of association between outcome and exposure variables. A p-value less than 0.05 was considered statistically significant.

4.3: Results

4.3.1: Duration of pre-patent period for *S. mansoni* in snails

The pre-patent period for *S. mansoni* was shorter in *B. pfeifferi* than in *B. sudanica* (Figure. 4.1). For all *S. mansoni* - *B. pfeifferi* combinations, some snails were shedding cercariae by 4 weeks PE, with sympatric combinations having higher proportions of early shedders (3% - 5.8%) compared to allopatric combinations (1.4% - 1.5% shedders). Allopatric combinations showed higher proportions of snails beginning shedding at 6 weeks PE. For *B. sudanica*, except for young adults exposed to sympatric *S. mansoni*, none of the other groups had shed by 4 weeks PE, and only a small percentage (1.2% – 3.4%) shed by 5 weeks PE. Even for juvenile *B. sudanica*, it took up to 6 weeks for shedding to commence. There was no obvious overall tendency for younger snails to shed earlier than older snails.

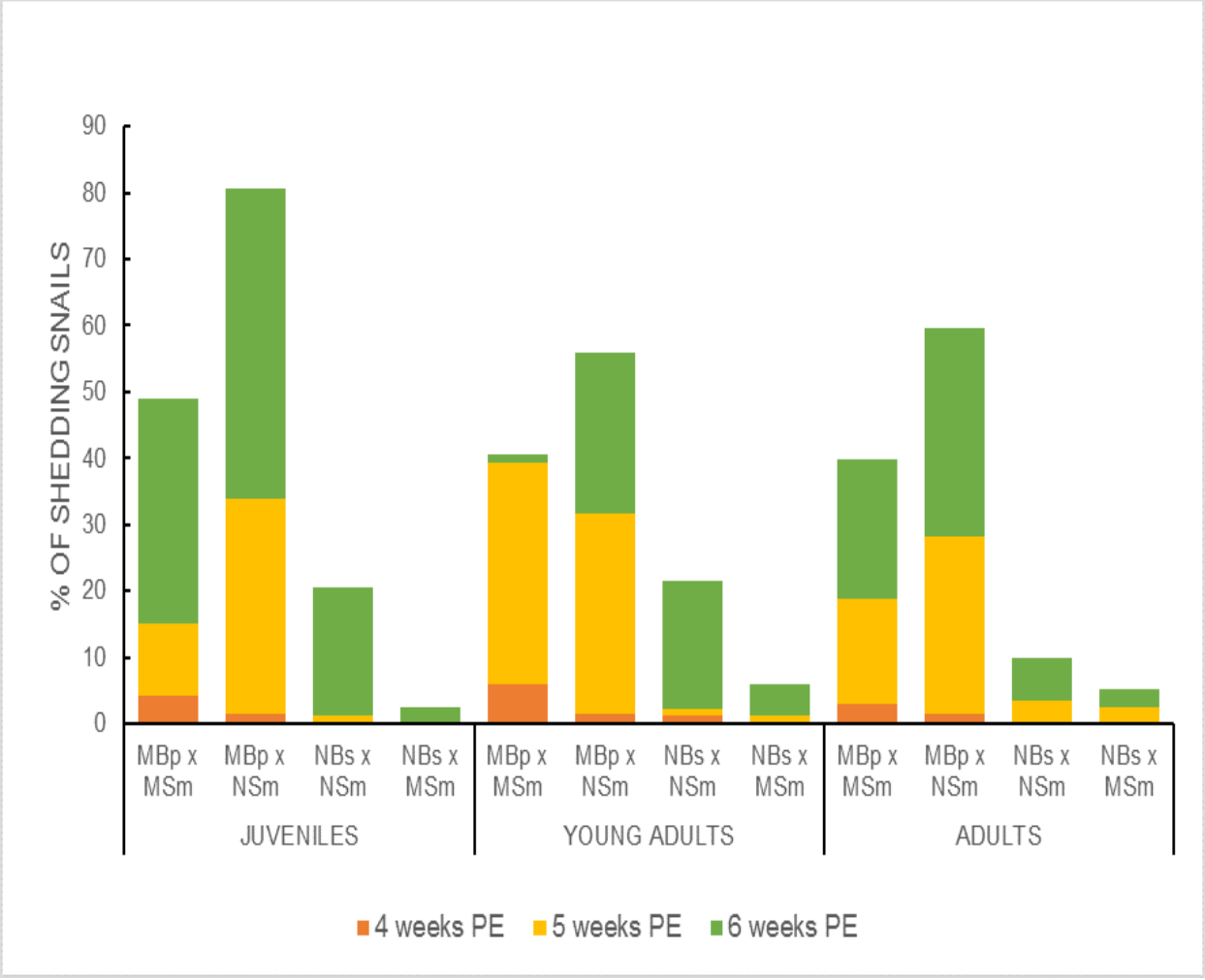


Figure 4.1: Percentage of snails in the different age categories shedding cercariae of *S. mansoni* from Nawa or Mwea at 4, 5, 6 weeks post-exposure (PE) to single schistosome miracidium. NBs represents *B. sudanica* Nawa, MBp, *B. pfeifferi* Mwea, MSm, *S. mansoni* Mwea, NSm, *S. mansoni* Nawa.

4.3.2: Prevalence of *S. mansoni* infection in snails

With respect to infection prevalence as measured by shedding of cercariae, snails in each exposure combination attained their peak prevalence of infection at 6 weeks PE, with an overall 30.6% prevalence among the 755 surviving exposed snails. *B. sudanica* were significantly less likely to develop cercariae-producing infections (48 of 414 surviving snails or 8.6%) than *B. pfeifferi* (183

of 341 surviving snails or 53.7%), regardless of the size of snails used or the source of *S. mansoni* (Figure 4.1; Table 4.1). For *B. sudanica* of all 3 size/age groups, the sympatric *B. sudanica*-transmitted Nawa *S. mansoni* isolate produced marginally higher prevalence of infection than the allopatric *B. pfeifferi* transmitted *S. mansoni* isolate from Mwea. The opposite was true for *B. pfeifferi* where the allopatric Nawa isolate of *S. mansoni* achieved higher prevalence levels than the sympatric Mwea isolate (significant for juvenile snails). In other words, for both snail species, and for all 3 size/age classes, the Nawa derived *S. mansoni* isolate always produced more patent infections than the Mwea derived *S. mansoni* isolate. The overall percentage of infection achieved among all snails exposed to Nawa *S. mansoni* (145 of 369 or 39.3%) was significantly higher ($p = 0.0078$) than for *S. mansoni* derived from Mwea (86 of 386 snails or 22.3%) (Figure 4.1; Table 4.1). Overall, relative to the juvenile snail prevalence level of 32.7%, infection prevalence of young adult and adult snails were not significantly different (27.7%, OR = 0.79 [95% CI = 0.55 – 1.13]; $p = 0.1938$), and (31.8%, OR = 0.96 [95% CI = 0.64 – 1.42]; $p = 0.8213$), respectively (Table 4.1).

Table 4.1: Analysis of *B. sudanica* Nawa and *B. pfeiferi* Mwea infections with *S. mansoni* (Nawa or Mwea) 6 weeks post exposure to single miracidium. OR= Odds ratio, CI= Confidence interval, UD=Undefined, Bs= *B. sudanica*, Bp= *B. pfeifferi*, Sm= *S. mansoni*

Snails category	Infected		Not infected		OR*	95% CI†		p Value
	n	%	n	%		Lower	Upper	
< 6mm shell diameter (Juveniles)								
Nawa Bs negative control	0	0.00%	90	100.00%	UD	UD	UD	
Mwea Bp negative control	0	0.00%	63	100.00%	UD	UD	UD	
Nawa Bs x Mwea Sm	2	2.40%	81	97.60%	1.00			
Nawa Bs x Nawa Sm	18	20.50%	70	79.50%	10.41	2.33	46.46	0.0021
Mwea Bp x Nawa Sm	46	80.70%	11	19.30%	169.36	35.96	797.56	<0.0001
Mwea Bp x Mwea Sm	26	49.10%	27	50.90%	39.00	8.68	175.27	<0.0001
Total	92	**32.70%**	189	67.30%				
6 - 9 mm shell diameter (Young adults)								
Nawa Bs negative control	0	0.00%	86	100.00%	UD	UD	UD	
Mwea Bp negative control	0	0.00%	77	100.00%	UD	UD	UD	
Nawa Bs x Mwea Sm	5	5.90%	80	94.10%	1.00			
Nawa Bs x Nawa Sm	17	21.50%	62	79.50%	4.39	1.53	12.55	<0.0001
Mwea Bp x Nawa Sm	29	55.80%	23	44.20%	20.17	7.02	58.02	<0.0001
Mwea Bp x Mwea Sm	28	40.60%	41	59.40%	10.93	3.93	30.40	<0.0001
Total	79	**27.70%**	**206**	**72.70%**				
> 9mm shell diameter (Adults)								
Nawa Bs negative control	0	0.00%	71	100.00%	UD	UD	UD	
Mwea Bp negative control	0	0.00%	67	100.00%	UD	UD	UD	
Nawa Bs x Mwea Sm	2	5.30%	36	94.70%	1.00			
Nawa Bs x Nawa Sm	4	9.80%	37	90.20%	1.95	0.34	11.29	0.458
Mwea Bp x Nawa Sm	31	59.70%	21	40.30%	26.57	5.77	122.45	<0.0001
Mwea Bp x Mwea Sm	23	39.70%	35	60.30%	11.83	2.59	53.97	0.0014
Total	60	31.80%	129	68.20%				
Overall								
Nawa Bs negative control	0	0.00%	247	100.00%	UD	UD	UD	
Mwea Bp negative control	0	0.00%	207	100.00%	UD	UD	UD	
Juveniles	92	32.70%	189	67.30%	1.00			
Young adults	79	27.70%	206	72.30%	0.79	0.55	1.13	0.1938
Old adults	60	31.80%	129	68.20%	0.96	0.64	1.42	0.8213
Total	231	30.60%	524	69.40%				

4.3.3: Snail mortality by 10 weeks post-exposure to *S. mansoni*

Except for the adult snails, mortality was higher for *B. pfeifferi* than *B. sudanica*, regardless of infection status (Figure 4.2; Table 4.2). Overall, relative to juvenile exposed snails, mortality among the unexposed control snails for both *B. sudanica* and *B. pfeifferi* was significantly lower at 20.0%, (OR = 0.16 [95% CI = 0.12 – 0.23]; p < 0.0001) and 36.0%, (OR = 0.37 [95% CI = 0.27 – 0.51]; p < 0.0001), respectively. For the miracidium exposed snails, relative to juvenile snails, young adult snails were 41% less likely to die, however, there was no significant increase in mortality for the adult snails when all the snails were considered together 62.20% (OR=1.11 [95% CI = 0.84 – 1.48]; p < 0.4686).

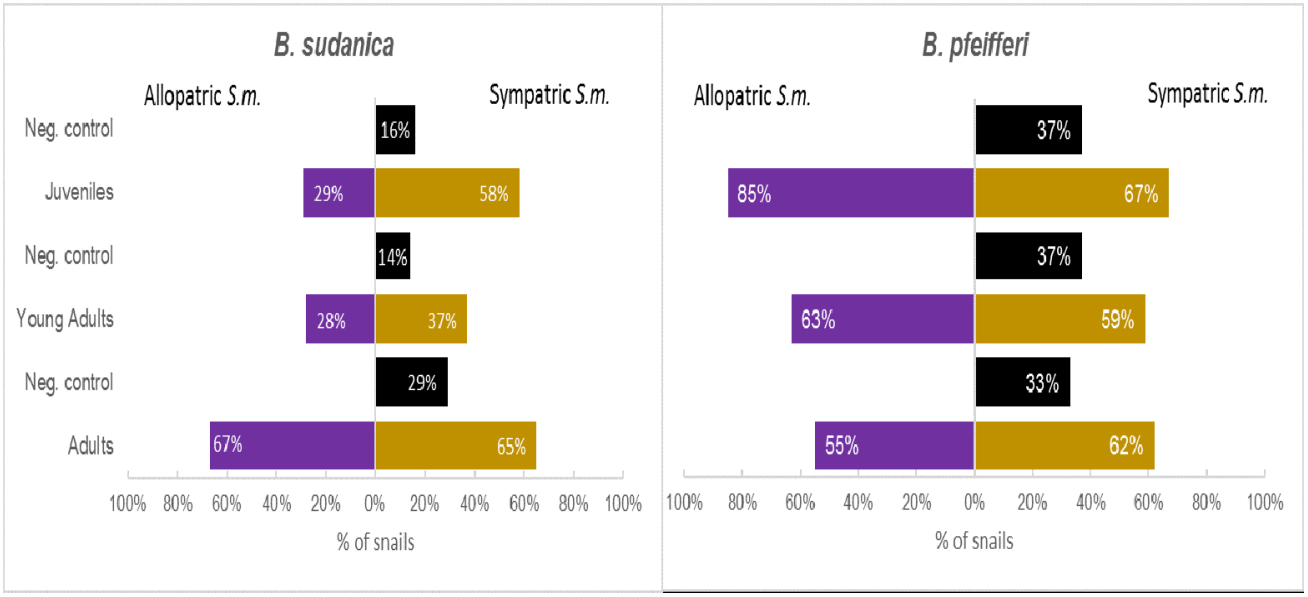


Figure 4. 2: Mortality rates for different snail-parasite combinations, at 10 wk post exposure. Black bars represent mortality rate for unexposed control groups for each of the corresponding snail categories. Snail mortality is expressed as a percentage of the initial number of snails in a combination; Neg. control = Negative (Unexposed control), *S.m* = *S. mansoni*.

Table 4.2: Analysis of mortality of the different categories of *B. sudanica* Nawa and *B. pfeiferi* Mwea 10 weeks post exposure to miracidia of *S. mansoni* (Nawa or Mwea) OR= Odds ratio, CI= Confidence interval, Bs= *B. sudanica*, Bp= *B. pfeifferi*, Sm= *S. mansoni*.

Snails category	Dead		Alive		OR*	95% CI†		p value
	n	%	n	%		Lower	Upper	
< 6mm shell diameter (Juveniles)								
Nawa Bs negative control	16	16%	84	84%	0.47	0.23	0.93	0.0296
Mwea Bp negative control	37	37%	63	63%	1.44	0.79	2.6	0.2298
Nawa Bs x Mwea Sm	29	29%	71	71%	1			
Nawa Bs x Nawa Sm	58	58%	42	42%	3.38	1.88	6.08	<0.0001
Mwea Bp x Nawa Sm	85	85%	15	15%	13.87	6.9	27.9	<0.0001
Mwea Bp x Mwea Sm	67	67%	33	33%	4.97	2.73	9.06	<0.0001
Total	292	48.67%	308	51.33%				
6 - 9 mm shell diameter (Young adults)								
Nawa Bs negative control	14	14%	86	86%	0.42	0.21	0.85	0.0168
Mwea Bp negative control	37	37%	63	63%	1.51	0.83	2.74	0.1753
Nawa Bs x Mwea Sm	28	28%	72	72%	1			
Nawa Bs x Nawa Sm	37	37%	63	63%	1.51	0.83	2.74	0.1753
Mwea Bp x Nawa Sm	63	63%	37	37%	4.38	2.41	7.95	<0.0001
Mwea Bp x Mwea Sm	59	59%	41	41%	3.7	2.05	6.68	<0.0001
Total	238	39.67%	362	60.33%				
> 9mm shell diameter (Adults)								
Nawa Bs negative control	29	29%	71	71%	0.2	0.11	0.37	<0.0001
Mwea Bp negative control	33	33%	67	67%	0.24	0.13	0.44	<0.0001
Nawa Bs x Mwea Sm	67	67%	33	33%	1			
Nawa Bs x Nawa Sm	65	65%	35	35%	0.91	0.51	1.64	0.7653
Mwea Bp x Nawa Sm	55	55%	45	45%	0.6	0.34	1.07	0.0829
Mwea Bp x Mwea Sm	62	62%	38	38%	0.8	0.45	1.44	0.4603
Total	311	51.83%	289	48.17%				
Overall								
Nawa Bs negative control	59	20%	241	80%	0.16	0.12	0.23	<0.0001
Mwea Bp negative control	107	36%	193	64%	0.37	0.27	0.51	<0.0001
Juveniles	239	59.80%	161	40.20%	1			
Young adults	187	46.80%	213	53.20%	0.59	0.45	0.78	0.0002
Old adults	249	62.20%	151	37.80%	1.11	0.84	1.48	0.4686
Total	841	46.72%	959	53.28%				

*-Odds Ratio, †- 95% Confidence Interval, Bs= *B. sudanica*, Bp= *B. pfeifferi*, Sm= *S. mansoni*

4.3.4: Survival of snails with *S. mansoni* cercariae infections.

For snails shedding *S. mansoni* cercariae (Table 4.3), except for the adult snails, *B. sudanica* had higher mean survival time compared with *B. pfeifferi*, with *B. sudanica* exposed to sympatric *S. mansoni* achieving slightly longer mean survival times (9.5 – 12.2 weeks) than with allopatric *S. mansoni* (9.5 – 11.0 weeks). However, whereas no infected *B. sudanica* survived past 29 weeks PE, a small percentage (1.5%) of young and adult *B. pfeifferi* exposed to sympatric *S. mansoni* survived for up to 40 weeks PE. By comparison, the longest recorded survival of unexposed *B. sudanica* and *B. pfeifferi* was 43 and 49 weeks, respectively.

Table 4.3: Mean survival time for *S. mansoni* cercariae shedding snails. Bs= *B. sudanica*, Bp= *B. pfeifferi*, Sm= *S. mansoni*

Snail - parasite Combination	Mean survival times (Weeks)	Standard error	Confidence interval (95%)	
			Lower	Upper
Juveniles				
Mwea Bp X Mwea Sm	10.9	0.6	9.7	12.1
Mwea Bp X Nawa Sm	9.3	0.4	8.6	10.1
Nawa Bs X Nawa Sm	11.8	0.9	10.0	13.6
Nawa Bs X Mwea Sm	10.5	1.5	7.5	13.5
Young adults				
Mwea Bp X Mwea Sm	10.1	0.8	8.5	11.8
Mwea Bp X Nawa Sm	10.6	0.8	9.0	12.1
Nawa Bs X Nawa Sm	12.2	0.5	11.1	13.2
Nawa Bs X Mwea Sm	11.0	0.4	10.1	11.9
Adults				
Mwea Bp X Mwea Sm	13.7	1.3	11.1	16.3
Mwea Bp X Nawa Sm	11.7	0.4	10.8	12.5
Nawa Bs X Nawa Sm	9.5	0.9	7.8	11.2
Nawa Bs X Mwea Sm	9.5	2.5	4.5	14.5

4.3.5: Cercariae production

Overall, infected *B. pfeifferi* produced more cercariae than *B. sudanica* with almost all the mean counts for the former being higher than the latter species (Figure 4.3; Table 4.4). For *B. pfeifferi*, sympatric combinations usually had a higher mean cercariae production (583 [95% CI 404-762] – 1,686 [95% CI 886-2,486]) than allopatric combinations (392 [95% CI 255-529] -1,232 [95% CI 936-1,528]). There was no obvious trend for smaller snails to produce fewer cercariae than bigger/older snails, but the highest counts did come from young adult or adult snails. There was no obvious overall tendency for cercariae production to either increase or decrease over 3 successive periods of observation spread over an interval of 56 days. The highest number of cercariae produced at a single observation time was 4,460 by an adult *B. pfeifferi* exposed to sympatric *S. mansoni.*

For *B. sudanica*, generally more cercariae were produced by snails with sympatric than allopatric *S. mansoni* infections (161 [95% CI 103-218] – 360 [95% CI 279-441]) but again this was not always the case. In these monomiracidial infections, no *B. sudanica* snails produced over 1,000 cercariae during the observation period though this was common with infected *B. pfeifferi* with sympatric *S. mansoni*.

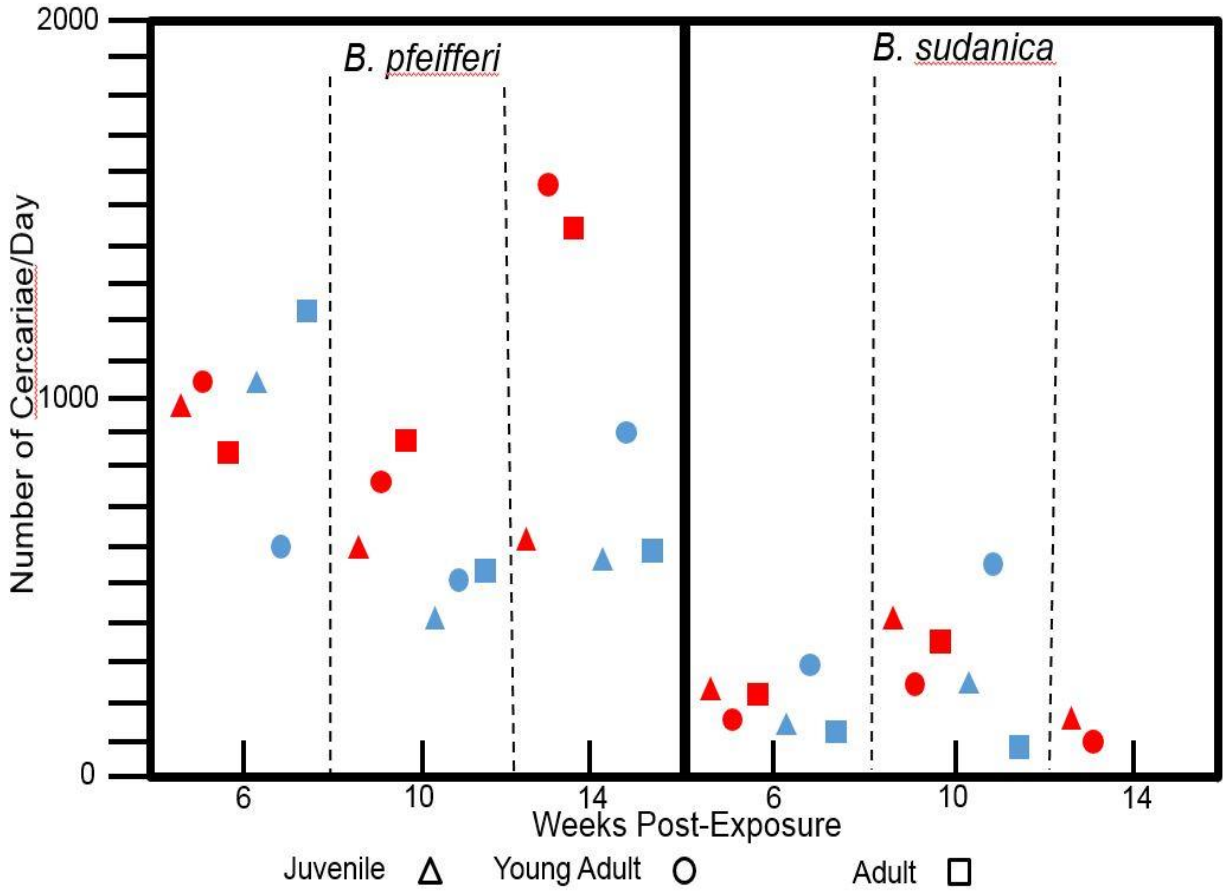


Figure 4.3: Mean cercariae produced by *B. pfeifferi* and *B. sudanica* at 6, 10 and 14 weeks post exposure. Sympatric combinations are represented in red and allopatric combinations in blue.

Table 4.4: Analysis of mean cercariae production within each snail-parasite combination at 6, 10 and 14 weeks post exposure

Snail size	Snail species	Snail source	Parasite source	Number of shedding snails			Mean cercariae output for 2 hours (95% CI)			P - Value
				6 weeks PE	10 weeks PE	14 weeks PE	6 weeks PE	10 weeks PE	14 weeks PE	
Juveniles	*B. sudanica*	Nawa	Nawa	17	7	4	258 (146-370)	451 (274-628)	176 (27-325)	0.0627
	B. sudanica	Nawa	Mwea	2	1	0	150 (51-249)	250		0.2207
	B. pfeifferi	Mwea	Mwea	25	10	5	984 (789-1,179)	583 (404-762)	624 (454-794)	0.015
	B. pfeifferi	Mwea	Nawa	46	10	2	1,032 (862-1,201)	392 (255-529)	568 (306-829)	0.0009
Young adults	*B. sudanica*	Nawa	Nawa	17	11	1	161(103-218)	250 (173-328)	60	0.0261
	B. sudanica	Nawa	Mwea	5	3	0	310 (48-572)	590 (243-937)		0.1797
	B. pfeifferi	Mwea	Mwea	28	14	5	1,051 (814 – 1,288)	760 (489-1031)	1,686 (886-2,486)	0.0425
	B. pfeifferi	Mwea	Nawa	29	9	8	607 (442-772)	513 (288-739)	880 (660-1100)	0.1033
Adults	*B. sudanica*	Nawa	Nawa	4	2	0	227 (81-374)	360 (279-441)		0.1649
	B. sudanica	Nawa	Mwea	2	1	0	120 (40-200)	50		0.2207
	B. pfeifferi	Mwea	Mwea	23	15	10	851 (628-1,073)	859 (613-1104)	1,476 (782-2,170)	0.0736
	B. pfeifferi	Mwea	Nawa	30	23	1	1,232 (936-1,528)	535 (379-691)	585	0.0016

4.4: Discussion

Following exposure to *S. mansoni,* for *B. pfeifferi* relative to *B. sudanica*, pre-patent period tended to be shorter, the prevalence of infection as measured by shedding significantly higher, the mortality at 10 weeks higher, average survivorship of infected snails marginally shorter, and the average daily production of cercariae significantly higher. In general, the size/age of the snails exposed (<6, 6-9, and >9mm shell diameter) did not strongly affect most parameters studied for either snail species.

Following exposure to a single miracidium, field isolates of *B. pfeifferi* consistently attained a prevalence of infection of 40+%. This was noted to be the case whether *S. mansoni* is derived from locations sympatric to the *B. pfeifferi* isolate (both for Asao stream in west Kenya and now observed twice for *B. pfeifferi* from canals in the Mwea rice scheme in central Kenya), or from allopatric locations (Mutuku *et al*. 2014). High susceptibility of *B. pfeifferi* to allopatric *S. mansoni* isolates has been shown to be the case regardless of whether the *S. mansoni* isolates come from regions in which *B. pfeifferi* is the usual host or from regions where *B. sudanica* is the normal host. For instance, 80% of juvenile *B. pfeifferi* from Mwea in central Kenya became infected following exposure to a single miracidum of *S. mansoni* from Nawa, Lake Victoria, approximately 300km to the west. this results are in agreement with most but not all previous studies (Frandsen, 1979; Southgate *et al*., 2000; Ibikounlé *et al.*, 2012; Adriko *et al*., 2013; Lu *et al*., 2016) in documenting high levels of compatibility of *S. mansoni* with both sympatric and allopatric *B. pfeifferi*, including those in which snail and schistosome originated from different continents (Frandsen, 1979; Ibikounlé *et al*., 2012).

In contrast to Adriko *et al*., (2013) and in agreement with Frandsen (1979), the results consistently show lower levels of success for *S. mansoni* in *B. sudanica*, in either sympatric or allopatric combinations. The Nawa isolate of *S. mansoni*, which was recovered from individuals from the shores of Lake Victoria, where their infections most probably originated from *B. sudanica*, never infected more than 25% of sympatric *B. sudanica*, even though this same isolate proved to be very compatible with allopatric *B. pfeifferi* (80% prevalence). Comparing the responses to *S. mansoni* of laboratory reared *B. sudanica* and field-derived *B. pfeifferi* using a combination of an *S. mansoni*-specific PCR-based detection assay, dissection and shedding methods (Lu *et al*., 2016), it was noted that more *B. pfeifferi* (54.5%) than *B. sudanica* (38.9%) were positive for *S. mansoni* at 1-4 days PE. This suggested that penetration of miracidia was somewhat higher for *S. mansoni* in *B. pfeifferi*, by 8-24 days PE, the proportion of dissection positive/PCR positive snails was over 2X higher in *B. pfeifferi* than in *B. sudanica*. By 40 days PE, the proportion of all snails that was unequivocally positive for *S. mansoni* was 3.2X higher and the proportion of all snails that was shedding was over 12X higher than for *B. sudanica*. *S. mansoni* also developed faster in *B. pfeifferi* than in *B. sudanica* (Lu *et al*., 2016).

Also, of considerable relevance to understanding the capacity of snails to transmit schistosomiasis is their longevity, especially their duration of shedding. It was observed that *B. pfeifferi* of all age groups, both unexposed controls and exposed snails, suffered high mortality by 10 weeks PE, generally higher than seen for *B. sudanica*. Exposure combinations with high prevalence of infection had particularly high mortality by 10 weeks PE, so this provides some explanation for the high mortality observed. Another factor which cannot be exclude is that the *B. pfeifferi* used were transported from central to western Kenya and may have suffered both from transportation and different environmental conditions at Kisian. It was noted that some *B. pfeifferi* isolates adapt better

to laboratory conditions than others. In spite of the high mortality at 10 weeks PE, some *B. pfeifferi* nonetheless survived and continued to shed for up to 40 weeks PE. In contrast, none of the exposed *B. sudanica* survived past 29 weeks PE. As previously noted, (Mutuku *et al.,* 2014), some individual field-derived *B. pfeifferi* when experimentally exposed to *S. mansoni* as young adults can support production of *S. mansoni* cercariae for over a year.

With respect to the number of cercariae released per day, on average *B. pfeifferi* produced more cercariae than *B. sudanica*. For individual *B. pfeifferi*, >2,000 cercariae were often recovered from a 2-hr shedding period. By contrast, none of the experimentally exposed *B. sudanica* were observed to shed more than 900 cercariae in a comparable interval, in agreement with results by Frandsen (1979). Low infection and cercariae production levels help to explain the difficulties experienced in the past in trying to maintain the *S. mansoni* life cycle in the laboratory in *B. sudanica*. In contrast to results of this study, a study utilizing Ugandan *Biomphalaria* snail isolates demonstrated that *B. sudanica* produced more cercariae than *B. pfeifferi* though the difference was not significant (Adriko *et al.*, 2013).

The results of this study are also of relevance to evolutionary biologists interested in the Red Queen hypothesis, particularly the topic of how sexual reproduction in host populations influences susceptibility to coevolving parasites. Studies of other digenean-snail combinations have shown that high prevalence of digenean infection favors higher proportions of sexually reproducing snails. This is because the parasites are able to adapt readily to clonal asexual hosts and achieve high levels of sterilizing infection among them whereas host individuals produced by sexual crosses may express rare traits conferring higher resistance to infection (Howard *et al*., 1994; Vergara *et al.,* 2014; Gibson *et al*., 2016). With respect to this study, it was noted as several others have, that *B. pfeifferi* is highly susceptible to *S. mansoni*, and in nature is often infected with several digenetic

trematodes (Loker *et al.*, 1981; Mohammed *et al*., 2016). A number of studies have documented that although *B. pfeifferi* is capable of cross-fertilization, it is a strong preferential self-fertilizer (Jarne *et al*., 2001; Charbonnel *et al*., 2005; Campbell *et al*., 2010). The latter mode of reproduction generally leads to an excess of homozygosity and a loss of genetic diversity, and *B. pfeifferi* populations consist of a series of relatively demarcated lineages separated from one another by strong preferential selfing (Jarne *et al*., 2001). These are all characteristics that might be expected to favor high levels of parasitism. Currently, there is lack of general understanding in *S. mansoni* transmission foci for how many such *B. pfeifferi* lineages exist, how spatially distinct or temporally stable they are, and how much they may vary if at all with respect to susceptibility to *S. mansoni* or to the many other species of digenetic trematodes with which they must contend. These other digenean species can be as common or more so than *S. mansoni*, and also impose fitness costs as they also can cause castration (Esch and Fernandez, 1994; Lafferty *et al*., 2009). It will be interesting to learn if under heavy pressure from parasitism including *S. mansoni* and other abundant digeneans like amphistomes (Laidemitt *et al*., 2016) whether *B. pfeifferi* populations increase out-crossing rates such that they might then fare better in a co-evolutionary arms race with outcrossing parasites (King *et al*., 2011; Koskella *et al*., 2011; Singh *et al*., 2015). Alternatively, perhaps some genotypes of *B. pfeifferi* are more resistant to digeneans than others, and selective pressure from digenean parasitism may favor increased frequency of resistant genotypes. Although the impermanent nature of the stream habitats often colonized by *B. pfeifferi* may prevent stable parasite populations from building such that the presumed advantages in rapid colonizing ability favored by self-fertilization might predominate over advantages in resistance to parasites resulting from cross fertilization, studies suggest high levels of parasitism can persist for years in streams that do not necessarily flood or dry on an annual basis (M.R. Laidemitt, Pers. Comm.). Further

investigation of the interactions between digenean parasitism (including *S. mansoni*) and both outcrossing rates and population composition studies for *B. pfeifferi* are clearly warranted, including efforts to determine if outcrossed progeny enjoy greater resistance to digenean infection. *B. sudanica* and *B. choanomphala* also deserve consideration in a broader evolutionary context. *B. choanomphala* has been characterized as an out-crosser (Standley *et al*., 2014). In the context used by the authors, this designation was meant to apply to both *B. choanomphala* and *B. sudanica* given that other studies suggested the two regularly undergo genetic exchange (the 2 taxa represent distinctive ecophenotypes) and *B. choanomphala* is the name with taxonomic priority (Standley *et al*., 2011). Here the name *B. sudanica* rather than *B. choanomphala* is used to apply to the shore-inhabiting form, because the latter is generally considered to be a deep-water snail. In any case, the exact nature of the genetic exchange between the 2 named taxa deserves further study for additional populations. Also, assuming that the lakeshore-inhabiting form *B. sudanica* is an out-crosser, it is of interest and consistent with theory that its experimental susceptibility to infection with either sympatric or allopatric *S. mansoni* is lower than seen with *B. pfeifferi*. Furthermore, overall prevalence levels with *S. mansoni* and other digeneans appear to be lower in *B. sudanica* than in *B. pfeifferi* in natural habitats monitored for over 2 years (M. R. Laidemitt, pers. comm.) though extraneous environmental factors might play an important role in dictating such infection levels as well. Any gain in resistance achieved by *B. sudanica* by out-crossing might be expected to affect not just *S. mansoni*, but as many as 16 additional digenean species, many transmitted by shoreline-inhabiting birds, that commonly infect this snail in nature as well. Although experimentation with additional isolates is needed, the low infection levels retrieved with *B. sudanica* following experimental exposure to *S. mansoni* are suggestive of the presence in *B. sudanica* of resistance traits that may prove useful with respect to developing new control efforts based on introductions

of resistant snails into natural populations of schistosome-susceptible snails. Lastly, the relationships between *B. choanomphala* and digenean infection deserve much more scrutiny. This taxon is typically but not always recovered from deeper lake water. Deepwater habitats have been considered as coevolutionary "cold spots" as compared to shallower shoreline habitats frequented by avian definitive hosts (Howard *et al*., 1994; King *et al*., 2009). For *B. choanomphala*, does its preferred habitat provide a refugium from digenean infection? Although it is clear that *B. choanomphala* can be infected by *S. mansoni* in deeper water, the extent to which it is exposed to this and other digenean species may be considerably diminished relative to *B. sudanica*. If so, then does this taxon when in deep water revert to more frequent self-fertilization, possibly abandoning the costs of maintaining resistance to digenean infection given their lower exposure rates? (Sheldon and Verhulst, 1996).

Another observation of interest from this study is that *S. mansoni* from Nawa, where it is transmitted by *B. sudanica*, produced higher infection levels in both snail species than the *B. pfeifferi*-transmitted isolate of *S. mansoni* isolated from Mwea. This is consistent with the idea that ongoing coevolutionary interactions of *S. mansoni* with a sexually reproducing host confers on its properties of infectivity that guarantee it a higher likelihood of success when confronted with a selfing species like *B. pfeifferi*. Study of further reciprocal exposure experiments involving the same two snail species and isolates of *S. mansoni* derived from each would be of interest to further document this possibility. Also of interest would be to learn if and how the interactions between sexual versus selfing snails might also influence trade-offs that might occur with respect to virulence in the definitive host (Davies *et al*., 2001).

4.5: Conclusion

This study was aimed at determining which of the two most prominent intermediate host snails for *S. mansoni* in Kenya is more efficient in transmission of the intestinal schistosomiasis parasite by measuring traits that affect transmission. At least some *B. sudanica* and *B. pfeifferi* could support full development of either allopatrically or sympatrically derived *S. mansoni* regardless of snail size/age, but *B. pfeifferi* were significantly more likely to become infected and had higher daily rates of cercariae production than *B. sudanica*. Even though *B. sudanica* seems less efficient in transmission of the parasite on a per snail basis, this species occurs in vast numbers in its natural habitat. Abundance may thus compensate for low compatibility such that *B. sudanica* can readily sustain transmission in communities living around the shores of the lake. The persistence of a proportion of long-term *B. pfeifferi* shedders, though it may seem insignificant, could nonetheless play a significant role in initiating reinfections in the face of sustained mass drug administration. Because of differences in the breeding systems of *B. pfeifferi* and *B. sudanica*, the interactions of these 2 host species with *S. mansoni* and other digeneans may prove to be instructive in understanding the importance of cross fertlization in resistance to parasites.

CHAPTER FIVE: COMPARATIVE COMPATIBILITY OF *BIOMPHALARIA SUDANICA* AND *B. CHOANOMPHALA*, SNAIL INTERMEDIATE HOSTS OF *SCHISTOSOMA MANSONI* IN LAKE VICTORIA, WESTERN KENYA

5.1: Introduction

Intestinal schistosomiasis caused by *S. mansoni* continues to be a public health concern in the Lake Victoria basin in western Kenya despite various chemotherapy-based control efforts (Onkanga *et al*., 2016; Karanja *et al*., 2017). Within this region, the prevalence of *S. mansoni* is high on the islands in the lake and villages neighboring the lake shore, and seems to decline with increasing distance from the lake shore (Odiere *et al*., 2012; Samuels *et al*., 2012). Transmission of *S. mansoni* within the Lake Victoria basin is perpetuated by 3 *Biomphalaria* snail taxa which occupy distinct habitats (Brown, 1994; Dejong *et al*., 2001; Mutuku *et al*., 2019).

1) *B. pfeifferi*, an inhabitant of streams, canals, ponds and other small water impoundments.

2) *B. sudanica*, which lives in shallow waters on the shores of Lake Victoria and the surrounding swamps.

3) *B. choanomphala*, a deepwater snail that lives on the lake bottom, but is sometimes swept to the shore line by strong water currents.

Molecular studies of mitochondrial and nuclear markers of *B. sudanica* and *B. choanomphala* suggest that the two taxa are not highly divergent genetically, and should probably be considered as a single species (Standley *et al*., 2011; Zhang *et al*., 2018).

In a recent study which involved a four-year annual praziquantel (PZQ) treatment of school children in villages around the lake shore, it was observed that whereas in some villages, the

prevalence of *S. mansoni* decreased drastically to less than 30% at the end of the treatment campaign (responding (RESP) villages), in other villages, prevalence remained high and above 30% after the four year treatment campaign, and these were designated as persistent hotspots (PHS villages) (Wiegand *et al*., 2017). In a follow-up study, it was observed that in comparing RESP and PHS villages, there were no significant differences in relative abundance of *B. sudanica* in shoreline habitats sampled or prevalence of *S. mansoni* infection in the snail populations (Mutuku *et al*., 2019). However, *B. choanomphala* was recovered from all of the PHS villages which were located in the west facing shoreline of the lake, and only from one of the RESP village that was located adjacent to the PHS villages. The deeper water *B. choanomphala* was significantly more abundant in the PHS villages and prevalence of *S. mansoni* among villages both before and after control was positively correlated with *B. choanomphala* abundance (Mutuku *et al*., 2019). It is worth noting that the villages were located in two distinct habitats with the RESP villages being located along the shores of the Winam Gulf, and the PHS villages on the shores of the open waters of the west facing shoreline; this could present ecological differences that could explain the differential presence of *B. choanomphala*. Of the total *Biomphalaria* snails retrieved from the PHS villages that were infected with *S. mansoni*, 3.5% were *B. choanompahala.* In a different study done in Mwanza, Tanzania, further south on the shore of L. Victoria, 12.2% of all *Biomphalaria* positive for *S. mansoni* were *B. choanomphala* (Gouvras *et al*., 2017). Both studies indicate that although most transmission occurs via the shore-inhabiting *B. sudanica*, *B. choanomphala* too plays an underestimated role in the transmission of schistosomiasis in the Lake Victoria basin. In both these studies, there was no obvious indication that *B. pfeifferi* played a role in *S. mansoni* transmission in the lake.

A key determinant of schistosomiasis transmission success is compatibility of the local snail population with schistosome infection. Compatibility as defined here has several components including the likelihood that exposure of a snail to a miracidium or miracidia leads to a cercariae-producing infection. The greater the compatibility, the more snail infections expected to result from a given level of schistosome egg input into the habitat (Anderson *et al*., 1979; French *et al*., 2010). Another key factor in compatibility of snails as intermediate host is the length of time required to complete sporocyst development for the first release of cercariae following exposure to miracidial infections (the pre-patent period). The longer the prepatent period, the more likely the infected snail might be to suffer mortality and never bring an infection to culmination. The remaining key factor in compatibility we consider is the daily and/or total output of cercariae produced by infected snails (Ibikounlé *et al*., 2012). The degree of compatibility can also be influenced strongly by whether the parasite and host derive from the same environment (are sympatric), or if host snails are exposed to *S. mansoni* miracidia from distant environments (allopatric combinations) (Adriko *et al*., 2013; Ibikounl´e *et al*., 2012: Mutuku *et al*., 2017; Mutuku *et al*., 2014; Southgate *et al*., 2012). Several studies have determined the vectorial competence of snail species that transmit *S. mansoni* in the East African region. A study of Kenyan field-derived *B. sudanica* and *B. pfeifferi* using *S. mansoni* derived from allopatric or sympatric sources established that *S. mansoni* developed faster and consistently had higher infection rates in *B. pfeifferi* (39.6-80.7%) than in *B. sudanica* (2.4-21.5%), regardless of the source of *S. mansoni* (Mutuku *et al.,* 2017). Mean daily cercariae production was greater for *B. pfeifferi* exposed to sympatric than for the snails exposed to allopatric *S. mansoni* (583–1,686 versus 392–1,232 respectively). Furthermore, mean daily cercariae output amongst *B. sudanica* was consistently low (50–590) with no significant differences between sympatric or allopatric combinations. In another study using Ugandan

Biomphalaria snail isolates, *B. sudanica* produced more cercariae than *B. pfeifferi*, even though the difference in cercariae output between the two species was not significant (Adriko *et al.*, 2013). Although experimentation with additional isolates is needed, the low measures of compatibility retrieved with *B. sudanica* following experimental exposure to *S. mansoni* in our hands are suggestive of the presence in *B. sudanica* of resistance traits that may affect force of transmission of the parasite to the definitive host.

The present study sought to examine the relative compatibility of *S. mansoni* with *B. sudanica* and *B. choanomphala*, the two snail taxa responsible for transmission of intestinal schistosomiasis to human populations living near the lake shore. The objective was to determine if the two snail hosts differ in their susceptibility to *S. mansoni* infection derived from a sympatric locality (one whose transmission depends on either *B. choanomphala or B. sudanica)* compared to allopatrically sourced *S. mansoni*, (one collected from a different location where it depends on *B. pfeifferi* for transmission). The study also, sought to determine daily cercariae output in the 2 snail taxa, another key parameter associated with vectorial competence.

5.2: Materials and Methods

5.2.1: Experimental design

A reciprocal cross infection experiment using first generation lab-reared snails was conducted in which *B. sudanica and B. choanomphala* snails were exposed to sympatric miracidia from Kanyibok or allopatric miracidia from Asao where transmission is usually perpetuated by *B. pfeifferi*. For each combination, 100 2-month old snails were exposed to either 1, 5 or 10 miracidia of *S. mansoni*. Another group of 100 snails for each of the 2 snail species were not exposed to the parasite, and served as negative controls. A total of 1,400 snails and 6,400 miracidia were used in

this experiment. Observations were made once a week over a period of at least 10 weeks. Snails were counted, screened for schistosome infections by the "shedding" method as described by Mutuku *et al*., 2014 starting from 3 weeks post exposure, and the number of snails surviving and those shedding cercariae, recorded. For snails that were found to be positive for schistosome infections, the total number of cercariae they produced within a 2 hr period was determined at 7 weeks post exposure.

5.2.2: Parasite and snail sources

S. mansoni eggs were obtained from pooled adult samples, 5 from each village; Asao, Nyakach sub-county, Kisumu County, western Kenya (00° 19' 01"S, 035° 00' 22"E) and Kanyibok, Bondo Sub-county, Siaya County, Western Kenya (00^{0}05'22.49"S, 034^{0}05'09.34"E). Schistosome eggs were concentrated and hatched, and miracidia used to infect snails as described by Mutuku *et al*. (2014). Initial snail populations of *B. sudanica* and *B. choanomphala* snails were collected by scooping and dredging respectively in Anyanga beach in Kanyibok as described in Chapter 3. The snails were then transported to the laboratory at the Center for Global Health Research (CGHR), KEMRI, Kisian, Kisumu, where they were sorted out into species, and screened individually for trematode infections. Any snails found to be shedding any type of cercariae were discarded.

5.2.3: Raising F1 generations of *B. sudanica* and *B. choanomphala*

The field derived *B. sudanica* or *B. choanomphala* snails were maintained for breeding in plastic aquaria measuring 60cm long x 30cm wide x 15cm deep in out-door, "semi-field" ambient conditions in a roofed, open-sided, screened structure at CGHR, KEMRI Kisian, as described in Chapter 3. The goal was to provide conditions that were close to what the snails would experience in the field, which ensure natural survival of the snails, and provide an environment conducive for snail breeding. After 1 month in the aquaria, the field-collected snails were removed, and the

upcoming F1 generation of juvenile snails were allowed to grow for another one month to young adults (shell diameter 6-9 and 5-7 mm for *B. sudanica* and *B. choanomphala* respectively) and then used in the experiment.

5.2.4: Determination of cercariae output from infected snails

Each snail was placed in an individual well of a 24-well plastic culture plate, containing 1ml of aged de-chlorinated tap water. The plate was placed in indirect sunlight for 2 hr between 10.00hr-12.00hr. Individual wells were then examined under a dissecting microscope for presence of cercariae. For the snails that had shed cercariae, the contents of the well were mixed gently using a micropipette, and an aliquot of 50 µL was then taken and placed in a gridded petri dish. Two drops of Lugol's iodine were then added to stain and immobilize the cercariae, and these were then counted with the aid of a dissecting microscope using a tally counter. The number of cercariae counted was multiplied by 20 to obtain the total number of cercariae that were produced by the snail during the 2 hr shedding period. This procedure was used for all the shedding snails at 7 weeks' post-exposure.

5.2.5: Ethical considerations

This study was approved by KEMRI's Scientific and Ethics Review Unit (SERU), and referenced SERU No.3540 and by the Institutional Review Board of the University of New Mexico (UNM), and referenced 18115. Recruitment of human study subjects, their participation and care were undertaken as described in Chapter 3. Consent to participate in the study was obtained in writing, after explaining the study to the participants, giving them opportunity to seek clarifications or ask further questions about the study, and addressing their concerns. Study participant information and data obtained from the participants were stored securely within KEMRI, on password-protected computers. This study was conducted with the approvals of the National Commission for Science,

Technology and Innovation (NACOSTI), Permit Number NACOSTI/P/16/9609/12754, and the National Environment Management Authority (NEMA), Permit Number NEMA/AGR/46/2014. Field collection of snails in the habitats was done under authorization from the Kenya Wildlife Service (KWS) permit number KWS/BRM/5001.

5.2.6: Statistical analyses

Descriptive statistics such as proportions were used to summarize categorical variables while measures of central tendency such as mean and range were used to summarize continuous variables. To determine effects of snail taxon and parasite source on infection status (yes/no) at the three different doses, a generalized linear model was used with a binomial distribution and logit link function due to the binary response variable. Thereafter, models were split by snail taxon in order to detect parasite source differences at three different doses within a tax on. For the latter individual taxon models, parasite source was nested within dose and dispersion was tested by a chi-squared test. Estimates and confidence intervals (CI) were back transformed to represent odd ratios. To determine whether snail taxon, parasite source and dose have an effect on the number of cercariae produced, a generalized linear model with a negative binomial distribution and a log link function were fitted to account for the response variable (number of cercariae) being count data and over dispersion (Kleiber and Zeileis, 2008). The “dispersion test” function from the AER package in R were used to detect over dispersion. Thereafter, models were split by taxon in order to determine whether there are any differences between parasite source within each taxon at three different doses. For the latter individual taxon models, parasite source was nested within dose.

5.3: Results

5.3.1: Pre-patent period for *S. mansoni* in snails

Overall pre-patent periods for *S. mansoni* in *B. sudanica* and *B. choanomphala* snails were not different with the majority of snails in all exposure combinations starting to shed cercariae by 5 weeks PE. By six weeks PE all the combinations attained their peak infection rates except for *B. choanomphala* exposed to 1 miracidium from Asao and *B. sudanica* exposed to 1 or 5 miracidia from Asao and 1 miracidium from Kanyibok. For these groups, all had 1 more shedding snail at 7 than 6 weeks post exposure.

5.3.2: Snail infection rates post exposure to *S. mansoni*

B. choanomphala had higher infection prevalence than *B. sudanica* at each dose, regardless of source of miracidia (Figure 5.1). As expected, none of the snails in the unexposed negative control groups became infected. For *B. choanomphala,* the sympatric combination (Kanyibok snails-Kanyibok parasite) generally yielded higher prevalence's, in the range 37.2-80.9%, than the allopatric combination (Kanyibok snails-Asao parasite) which produced prevalence's in the range 12.2-61.8%. For *B. sudanica*, prevalence's were consistently low, regardless of miracidial dose or source, with the sympatric combination producing marginally higher prevalence's of 7.5-18.6% relative to the allopatric combination which produced prevalence's of between 5.2-13.8%.

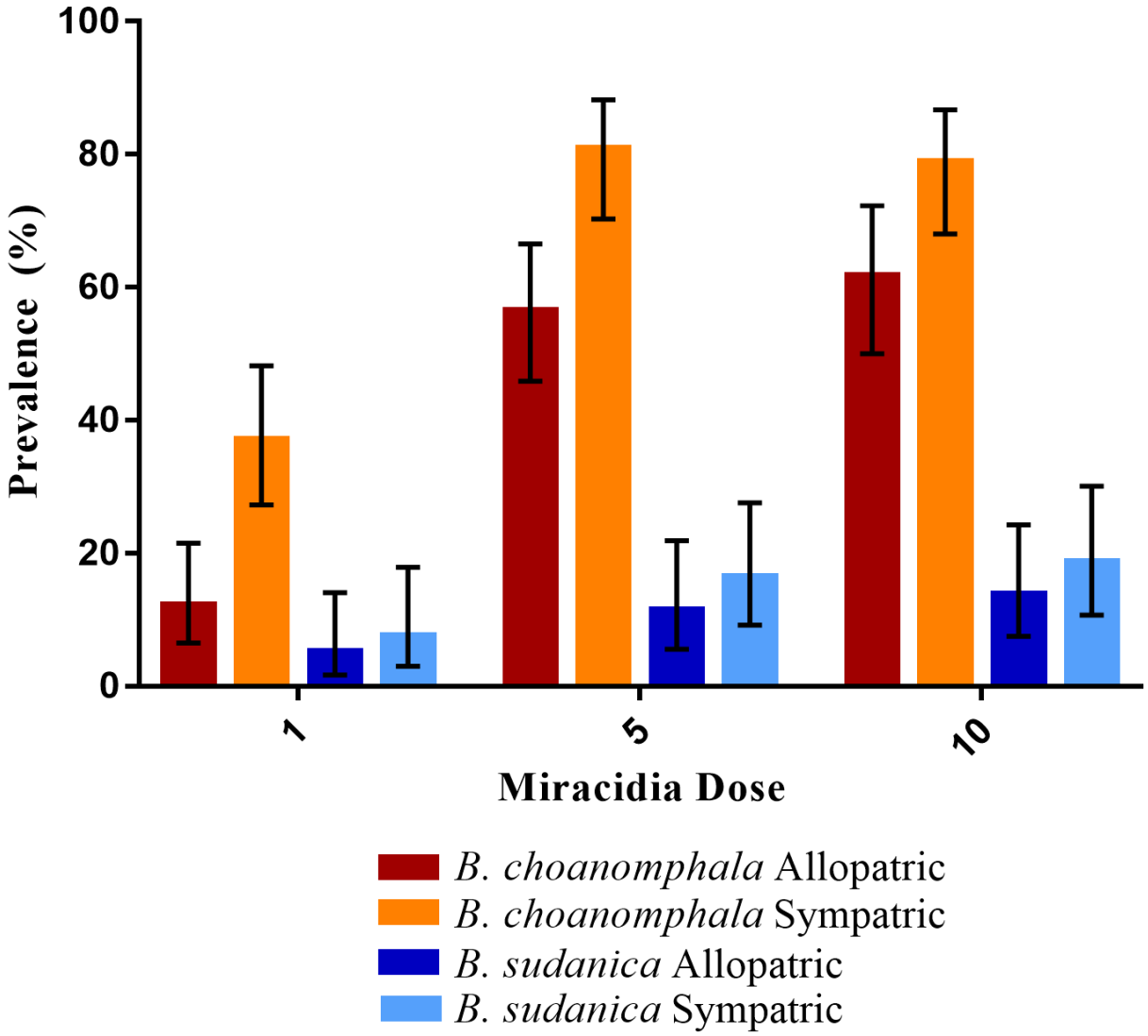


Figure 5.1: *S. mansoni* infection prevalence in *B. choanomphala* or *B. sudanica* 6 weeks after exposure to doses of 1, 5 or 10 miracidia from allopatric or sympatric combinations. Error bars represent 95% confidence interval.

5.3.3: Effect of snail species and parasite source on infection status of snails.

A summary of the generalized linear model with a binomial distribution and a link = logit function to determine whether or not snail taxon and parasite source have an effect on infection status is presented in Table 5.1. Overall, the odds of a snail becoming infected with 5 or 10 miracidia are higher (494 % and 569 % respectively) than the odds of being infected with 1 miracidium (GLM, Dose 5: β = 5.95, z = 7.65, P = <0.0001; Dose 10: β =6.69, z = 7.95, P = <0.0001), after accounting for snail taxon and parasite source. Although a dose of 10 miracidia had higher odds of infection than a dose of 5 miracidia, this difference was not statistically significant (GLM, β = 0.89, z = -0.58, P = 0.5620). *B. sudanica* have 92 % lower odds of being infected compared to *B. choanomphala*, after accounting for miracidia dose and source (GLM, β = 0.08, z = -12.05, P = <0.0001). The odds of infection with miracidia from an allopatric source is 59 % lower than infection with miracidia from a sympatric source, after accounting for species and dose (GLM, β = 0.41, z = -4.93, P = <0.0001).

Table 5.1: Summary of the generalized linear model with a binomial distribution and a link = logit function to determine whether the main effects of snail species and parasite source has an effect on infection status (yes/no) at three different doses (AIC = 787.6). Estimates and confidence intervals (CI) are back transformed to represent odd ratios. For interpretation purposes, the reference level is adjusted to dose 1 or 10 for the same model

	Reference level: dose = 1; taxon = *B. choanomphala*; source = sympatric			Reference level: dose = 10; taxon = *B. choanomphala*; source = sympatric		
Predictor	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value
Fixed effects						
Intercept (β_0)	0.55 (0.38-0.80)	-3.10	0.0019	3.70 (2.53-5.50)	6.61	<0.0001
Dose: 1	-	-	-	0.15 (0.09-0.24)	-7.95	<0.0001
Dose: 5	5.94 (3.79-9.45)	7.65	<0.0001	0.89 (0.59-1.33)	-0.58	0.5620
Dose: 10	6.69 (4.22-10.78)	7.95	<0.0001	-	-	-
Species: *B. sudanica*	0.08 (0.06-0.12)	-12.05	<0.0001	0.08 (0.06-0.12)	-12.05	<0.0001
Source: Allopatric	0.41 (0.28-0.58)	-4.93	<0.0001	0.41 (0.28-0.58)	-4.93	<0.0001

5.3.4: Effect of parasite source and miracidia dose on infection rates for *B. choanomphala*

With respect to *B. choanomphala* snails, the odds of becoming infected with 5 or 10 miracidia are 612 % and 531 % higher than the odds of being infected with 1 miracidia (GLM, Dose 5: β = 7.12, z = 5.19, P = <0.0001; Dose 10: β =6.31, z = 4.93, P = <0.0001), after accounting for parasite source, a dose of 5 miracidia had higher odds of infection than a dose of 10 miracidia, but this difference was not statistically significant (GLM, β = 1.13, z = 0.29, P = 0.7707). In contrast, *B. sudanica* had no significant differences between miracidia doses after accounting for parasite source (Table 5.3). *B. choanomphala* snails reduces the odds of infection by 77 %, 69 %, 57 % when exposed with 1, 5 and 10 miracidia respectively from an allopatric source compared to a sympatric source (GLM, Dose 1: β = 0.23, z = -3.45, P = 0.0006; Dose 5: β = 0.31, z = -3.19, P = 0.0014; Dose 10: β = 0.43, z = -2.19, P = 0.0288). Table 5.2 provides a summary of generalized linear models with a binomial distribution and a link = logit function for *B. choanomphala* to determine whether parasite source has an effect on infection status.

Table 5.2: Summary of generalized linear models with a binomial distribution and a link = logit function for *B. choanomphala* to determine whether parasite source has an effect on infection status (yes/no) at three different doses (AIC = 521.44). Estimates and confidence intervals (CI) are back transformed to represent odd ratios. For interpretation purposes, the reference level is adjusted to dose 1 or 10 for the same model.

	Reference level: dose = 1; source = sympatric			Reference level: dose = 10; source = sympatric		
Predictor	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value
Fixed effects						
Intercept (β_0)	0.59 (0.37-0.93)	-2.24	0.0252	3.73 (2.17-6.84)	4.53	<0.0001
Dose: 1	-	-	-	0.16 (0.07-0.32)	-4.93	<0.0001
Dose: 5	7.12 (3.47-15.38)	5.19	<0.0001	1.13 (0.50-2.57)	0.29	0.7707
Dose: 10	6.31 (3.10-13.45)	4.93	<0.0001	-	-	-
Interactions						
Dose (1): Source (Allopatric)	0.23 (0.10-0.51)	-3.45	0.0006	0.23 (0.10-0.51)	-3.45	0.0006
Dose (5): Source (Allopatric)	0.31 (0.15-0.62)	-3.19	0.0014	0.31 (0.15-0.62)	-3.19	0.0014
Dose (10): Source (Allopatric)	0.43 (0.20-0.91)	-2.19	0.0288	0.43 (0.20-0.91)	-2.19	0.0288

5.3.5: Effect of parasite source and miracidia dose on infection rates for *B. sudanica*

B. sudanica snails reduce the odds of infection by 33 %, 35 %, 30 % when exposed to 1, 5, and 10 miracidia from an allopatric source compared to a sympatric source, respectively, however these differences are not statistically significant (GLM, Dose 1: β = 0.67, z = -0.51 P = 0.6090; Dose 5: β = 0.65, z = -0.82, P = 0.4150; Dose 10: β = 0.70, z = -0.72, P = 0.4700, Table 5.3).

Table 5.3: Summary of the generalized linear models with a binomial distribution and a link = logit function for *B. sudanica* to determine whether parasite source has an effect on infection status (yes/no) at three different doses (AIC =271.16). Estimates and confidence intervals (CI) are back transformed to represent odd ratios. For interpretation purposes, the reference level is adjusted to dose 1 or 10 for the same model.

	Reference level: dose = 1; source = sympatric			Reference level: dose = 10; source = sympatric		
Predictor	β Estimate (2.5-97.5% *CI*)	***z***-value	*P*-value	β Estimate (2.5-97.5% *CI*)	***z***-value	*P*-value
Fixed effects						
Intercept (β_0)	0.08 (0.02-0.20)	-4.82	<0.0001	0.23 (0.11-0.42)	-4.41	<0.0001
Dose: 1	-	-	-	0.36 (0.09-1.12)	-1.67	0.0950
Dose: 5	2.40 (0.75-9.22)	1.40	0.1610	0.86 (0.33-2.21)	-0.32	0.7460
Dose: 10	2.81 (0.89-10.69)	1.67	0.0950	-	-	-
Interactions						
Dose (1): Source (Allopatric)	0.67 (0.13-3.17)	-0.51	0.6090	0.67 (0.13-3.17)	-0.51	0.6090
Dose (5): Source (Allopatric)	0.65 (0.22-1.82)	-0.82	0.4150	0.65 (0.22-1.82)	-0.82	0.4150
Dose (10): Source (Allopatric)	0.70 (0.26-1.83)	-0.72	0.4700	0.70 (0.26-1.83)	-0.72	0.4700

5.3.6: Number of cercariae produced by infected *B. sudanica* and *B. choanomphala* snails

On average, individual infected *B. choanomphala* produced more cercariae than *B. sudanica* (456 and 237.5 respectively). At low miracidia doses (1 and 5), *B. choanomphala* produced more cercariae in the sympatric combination, than in the allopatric combination. However, this was in contrast to *B. sudanica* which produced more cercariae when exposed to allopatric *S. mansoni*, compared with the sympatric combination (Figure 5.2). In general, for any particular combination of dose and miracidial source, fewer cercariae were produced by snails exposed to one miracidium.

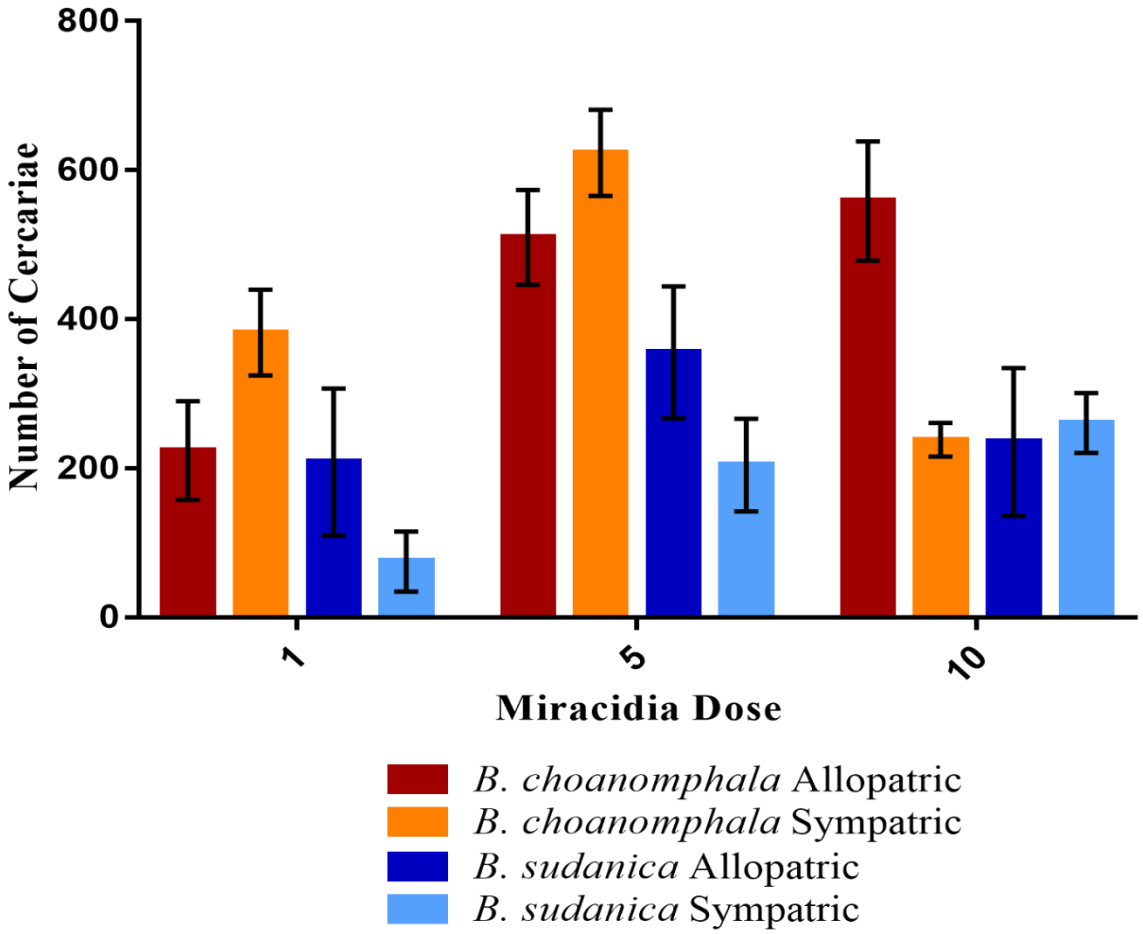


Figure 5.2: Average number of cercariae produced by snail taxon (*B. choanomphala* or *B. sudanica*) upon exposure to allopatric or sympatric *S. mansoni*, following 3 different miracidia doses (1, 5 and 10). Error bars represent standard error of the mean.

5.3.7: Effect of snail taxon, parasite source and miracidia dose on number of cercariae produced by snails

While accounting for snail taxon and parasite source, the expected number of cercariae from a dose of 5 miracidia are 65 % and 50 % higher than for doses of 1 and 10 miracidia, respectively (GLM, Dose 5: β_0 = 314.60, β = 1.65, z = 3.24, P = 0.0012; Dose 5: β_0 = 346.82, β = 1.50, z = 3.57, P = 0.0004. Interestingly, there was no statistically significant difference between the cercariae produced from miracidia doses of 1 and 10 (GLM, z = 0.63, P = 0.5298). The expected number of cercariae shed by *B. sudanica* is 46% lower than for *B. choanomphala*, after accounting for parasite dose and source (GLM, β = 0.54, z = -4.34, P = <0.0001).

After accounting for snail species and dose, the expected number of cercariae shed when they were exposed to an allopatric parasite is 23% higher compared to when they received parasites from a sympatric source (GLM, β = 1.98, z = -4.34, P = 0.0475). Table 5.4 provides results of the analysis.

Table 5.4: Summary of the effects of generalized linear model with a negative binomial distribution predicting whether snail taxon, parasite source and dose have an effect on cercariae produced by snails (AIC = 3989.1, Theta = 1.32). Estimates and confidence intervals (CI) are back transformed. For interpretation purposes, the reference level is adjusted to dose 1 or 10 for the same model.

	Reference level: dose = 1; taxon = *B. choanomphala*; source = sympatric			Reference level: dose = 10; taxon = *B. choanomphala*; source = sympatric		
Predictor	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value
Fixed effects						
Intercept (β_0)	314.60 (244.38-413.81)	42.10	<0.0001	346.82 (283.87-427.11)	61.31	<0.0001
Dose: 1	-	-	-	0.91 (0.67-1.25)	-0.63	0.5298
Dose: 5	1.65 (1.21-2.22)	3.24	0.0012	1.50 (1.19-1.87)	3.57	0.0004
Dose: 10	1.10 (0.80-1.50)	0.63	0.5298	-	-	-
Taxon: *B. sudanica*	0.54 (0.41-0.72)	-4.34	<0.0001	0.54 (0.41-0.72)	-4.34	<0.0001
Source: Allopatric	1.23 (1.00-1.53)	1.98	0.0475	1.23 (1.00-1.53)	1.98	0.0475

5.3.8: Effect of parasite source and miracidia dose on number of cercariae produced by *B. choanomphala*

The number of cercariae shed by *B. choanomphala* from a dose of 5 miracidia are 63 % and 161 % higher than a dose of 1 and 10 miracidia, respectively (GLM, Dose 5: β_0 = 382.32, β = 1.63, z = 2.58, P = 0.0099; Dose 5: β_0 = 238.48, β = 2.61, z = 6.23, P = 0.0004). Interestingly, *B. choanomphala* is expected to shed 60% more cercariae when exposed to a single miracidium compared to 10 miracidia (GLM, β = 1.60, z = 2.47, P = 0.0136). However, the number of cercariae shed within species are dependent on parasite source and dose. For example, *B. choanomphala* exposed to a dose of 10 allopatric miracidia shed 134 % more cercariae than sympatric miracidia at the same dose (GLM, β = 2.34, z = 5.05, P = <0.0001). In contrast, but not significantly different upon exposure to 1 or 5 allopatric miracidia, the number of cercariae produced reduced by 41 % and 18 %, respectively (GLM, Dose 1: β = 0.59, z = -1.69, P = 0.0914; Dose 5: β = 0.82, z = -1.24, P = 0.2142. Table 5.5 summarizes results of this analysis.

Table 5.5: Summary of generalized linear model with a negative binomial distribution predicting whether parasite source and miracidia dose have an effect on cercariae produced by *B. choanomphala* (AIC = 3397.6, Theta = 1.47). Estimates and confidence intervals (CI) are back transformed. For interpretation purposes, the reference level is adjusted to dose 1 or 10 for the same model.

	Reference level: dose = 1; source = sympatric			Reference level: dose = 10; source = sympatric		
Predictor	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value	β Estimate (2.5-97.5% *CI*)	*z*-value	*P*-value
Fixed effects						
Intercept (β_0)	382.32 (285.70-527.77)	38.08	<0.0001	238.48 (193.46-298.57)	49.52	<0.0001
Dose: 1	-	-	-	1.60 (1.11-2.35)	2.47	0.0136
Dose: 5	1.63 (1.11-2.35)	2.58	0.0099	2.61 (1.93-3.54)	6.23	0.0004
Dose: 10	0.62 (0.42-0.90)	-2.47	0.0136	-	-	-
Dose (1): Source (Allopatric)	0.59 (0.32-1.13)	-1.69	0.0914	0.59 (0.32-1.13)	-1.69	0.0914
Dose (5): Source (Allopatric)	0.82 (0.60-1.13)	-1.24	0.2142	0.82 (0.60-1.13)	-1.24	0.2142
Dose (10): Source (Allopatric)	2.34 (1.69-3.27)	5.05	<0.0001	2.34 (1.69-3.27)	5.05	<0.0001

5.3.9: Effect of parasite source and miracidia dose on number of cercariae produced by *B. sudanica*

For *B. sudanica*, the number of cercariae shed was not statistically significantly different when exposed to *S. mansoni* from either sympatric or allopatric source for the three different miracidia dose (1, 5 and 10). (GLM, Dose 1: $z = 1.51$, $P = 0.1304$; Dose 5: $z = 1.27$, $P = 0.2026$; Dose 10: $z = 0.90$, $P = 0.7965$; Table 5.6).

Table 5.6: Summary of generalized linear model with a negative binomial distribution predicting whether parasite source and infection dose have an effect on number of cercariae produced by *B. sudanica* (AIC = 576.52, Theta = 1.29). Estimates and confidence intervals (CI) are back transformed. For interpretation purposes, the reference level is adjusted to dose 1 or 10 for the same model.

	Reference level: dose = 1; source = sympatric			Reference level: dose = 10; source = sympatric		
Predictor	β Estimate (2.5-97.5% *CI*)	***z***-value	*P*-value	β Estimate (2.5-97.5% *CI*)	***z***-value	*P*-value
Fixed effects						
Intercept (β_0)	75.00 (34.96-206.17)	9.73	<0.0001	260.91 (161.47-461.37)	20.93	<0.0001
Dose: 1	-	-	-	0.29 (0.11-0.88)	-2.41	0.0159
Dose: 5	2.73 (0.88-7.23)	1.91	0.0556	0.78 (0.37-1.69)	-0.63	0.5273
Dose: 10	3.48 (1.14-9.05)	2.41	0.0159	-	-	-
Dose (1): Source (Allopatric)	2.78 (0.74-11.43)	1.51	0.1304	2.78 (0.74-11.43)	1.51	0.1304
Dose (5): Source (Allopatric)	1.74 (0.75-4.22)	1.27	0.2026	1.74 (0.75-4.22)	1.27	0.2026
Dose (10): Source (Allopatric)	0.90 (0.42-2.00)	-0.26	0.7965	0.90 (0.42-2.00)	-0.26	0.7965

Table 5.7: Summary of infection rates and mean cercariae produced upon exposure of *B. pfeifferi*, *B. sudanica* and *B. choanomphala* to various miracidia dose (Mutuku *et al*., 2014, 2017).

SUMMARY OF SNAILS INFECTION RATES UP ON EXPOSUR TO MIRACIDIA					
Mwea and Asao *B. pfeifferi* X mwea or Asao *S. mansoni* (2014)					
		B. pfeifferi (Mwea)		*B. pfeifferi* (Asao)	
Miracidia dose	Snail Size/age	Sympatric	Allopatric	Sympatric	Allopatric
1	Young adults	69.20%	45.50%	50.00%	43.60%
5	Young adults	93.80%	78.60%	48.70%	82.40%
10	Young adults	81.30%	100.00%	91.70%	83.30%
Mwea *B. pfeifferi* and Nawa *B. sudanica* X mwea or Nawa *S. mansoni* (2017)					
Miracidia dose		*B. pfeifferi* (Mwea)		*B. sudanica* (Nawa)	
	Snail size/age	Sympatric	Allopatric	Sympatric	Allopatric
1	Juveniles	49.10%	80.70%	20.50%	2.40%
1	Young adults	40.60%	55.80%	21.50%	5.90%
1	Adults	39.70%	59.70%	9.80%	5.30%
Kanyibok *B. choanomphala* and *B. sudanica* X Kanyibok or Asao *S. mansoni* (2019)					
Miracidia dose		*B. sudanica* (Kanyibok)		*B. choanomphala* (Kanyibok)	
	Snail size/age	Sympatric	Allopatric (Asao sm)	Sympatric	Allopatric (Asao Sm)
1	Young adults	7.55%	5.17%	37.18%	12.16%
5	Young adults	16.39%	11.48%	80.82%	56.47
10	Young adults	18.64%	13.85%	78.87%	61.76%
SUMMARY OF MEAN CERCARIAE PRODUCTION FROM 2 SUCCESIVE EXPERIMENTS					
Mwea *B. pfeifferi* and Nawa *B. sudanica* X mwea or Nawa *S. mansoni* (2017)					
Miracidia dose		B. pfeifferi (Mwea)		B. sudanica (Nawa)	
	Snail size/age	Sympatric	Allopatric	Sympatric	Allopatric
1	Juveniles	984	1,032	258	150
1	Young adults	1051	607	161	310
1	Adults	851	1,232	227	120
Kanyibok *B. choanomphala* and *B. sudanica* X Kanyibok or Asao *S. mansoni* (2019)					
Miracidia dose		*B. sudanica* (Kanyibok)		*B. choanomphala (Kanyibok)*	
	Snail size/age	Sympatric	Allopatric (Asao sm)	Sympatric	Allopatric (Asao Sm)
1	Young adults	75	208	382	224
5	Young adults	205	356	623	503
10	Young adults	261	236	238	559

5.4: Discussion

Throughout this study including in the following discussion, the names *B. sudanica* and *B. choanomphala* are used in part for convenience and in part in deference to the classical literature that recognizes them as distinct species (e.g. Brown, 1994). However, its noteworthy that there is good evidence that snails of the two taxa are very closely related, and probably should more correctly be considered as morphologically distinguishable forms of the same species (DeJong *et al*., 2001; Standley *et al*.,2011; Zhang *et al*., 2018). Which name, *sudanica* or *choanomphala,* deserves taxonomic precedence is an arguable point (Zhang *et al*., 2018). To avoid confusion in comparison of this study with others, its noted that Standley *et al* (2011) used "*B. choanomphala"* to refer to both forms from Lake Victoria. For the purposes of this study, it is noted that the form considered as "*choanomphala*" is the one usually dredged from deeper water, but is also occasionally recovered from shoreline populations, including at Kanyibok (Mutuku *et al*., 2019). It attains a smaller shell diameter and has distinct conchological features such as "strongly angular whorls beneath" and a small umbilicus, whereas the form typically referred to as "*sudanica*" found in shoreline populations and in adjacent papyrus swamps, has a larger diameter, flat shell with a wide umbilicus (Brown, 1994). It has been established that laboratory populations of both forms, both of which readily adapt to laboratory culture, retain their characteristic shell features.

In the present study comparing the compatibility of Kenyan *B. sudanica* and *B. choanomphala* to Kenyan *S. mansoni* using experimental infections, it has again been noted that *B. sudanica* has relatively low prevalence's of infection with *S. mansoni*, just as in previous studies comparing *B. sudanica* and *B. pfeifferi* (Table 5.7). For instance, in those studies it was noted infection prevalence's of (2.4-21.5%) for *B. sudanica* whereas *B. pfeifferi* had much higher experimental prevalence's (39.6-100%). The prevalence's achieved for *B. choanomphala* were closer to, but

generally lower than, those achieved with *B. pfeifferi* (Ibikounlé *et al*., 2012; Mutuku *et al*., 2014, 2017; Southgate *et al*., 2000). In contrast to *B. pfeifferi* which showed relatively high prevalence's of infection with both sympatric and allopatric isolates of *S. mansoni*, in this study, both *B. choanomphala* and *B. sudanica* tended to have higher prevalence's following exposure to sympatric *S. mansoni* isolates.

For both snail taxa, increasing the dose of miracidia generally increased the prevalence rate, but the response for *B. sudanica* was modest as compared to *B. choanomphala*. No combination of *S. mansoni* with either snail taxon achieved 100% prevalence. For a dose of one miracidium which is probably the most common exposure dose in nature, there was a significant difference ($P = 0.0006$) in infection rates between the two snail taxa with the odds of infection being 86 % lower for *B. sudanica* compared to *B. choanomphala* after accounting for species and dose. For *B. sudanica*, despite increasing ten-fold the number of parasite genotypes to which each snail was exposed, prevalence's increased only about two-fold and were lower than or comparable to the prevalence's achieved by exposure of *B. choanomphala* to a single miracidium. For *B. choanomphala*, although the prevalence rate increased substantially by 2-4-fold with an increase in dose to five miracidia, a further increase to ten miracidia did not have a large impact on further increasing the prevalence. The results suggest that a considerable fraction of *B. sudanica*, up to 80%, are for reasons yet to be determined refractory to *S. mansoni* infection. Likewise, there may be about 20% of *B. choanomphala* that are refractory even when exposed to 10 miracidia. In contrast, prevalence rates of over 90% and even in one case, 100%, were achieved with *B. pfeifferi* suggesting this species has a lower potential to be refractory. The basis for the refractory nature of *B. sudanica* deserves further study and may represent the presence of resistance genes that potentially could be exploited for control purposes.

With respect to other important components of compatibility, its noted that *B. sudanica* and *B. choanomphala* did not differ significantly with respect to length of the prepatent period (5-6 weeks for most snails exposed). In contrast, *B. pfeifferi* supports more rapid development of *S. mansoni*, in the range of 3-6 weeks (Adriko *et al*., 2013; Mutuku *et al*., 2014, 2017; Southgate *et al*., 2000). With respect to cercariae production, the expected number of cercariae shed by *B. sudanica* was 46 % lower than for *B. choanomphala*, carrying the implication that the risk per snail, posed by an infected *B. choanomphala* is higher than for an infected *B. sudanica*. Using similar methods, cercariae production was found to be substantially higher for *B. pfeifferi*, regardless of whether sympatric or allopatric combinations were studied (Table 5.7).

Considering the three components of compatibility together, for west Kenyan representatives of snails and *S. mansoni* in combination, *B. pfeifferi* was ranked as the most compatible, followed by *B. choanomphala*, then *B. sudanica*. Care is required in extending conclusions regarding compatibility to all parts of Lake Victoria or to other parts of the known ranges of the three snail taxa involved. For instance, Adriko *et al* (2013) working with F1 generation snails collected from the Ugandan shore of Lake Victoria, following exposure of *B. choanomphala* to 20 miracidia/snail, reported prevalence's less than 15% for both allopatric and sympatric combinations. They also reported relatively low compatibility levels for *B. pfeifferi*. Multiple factors including real biological differences among snails and parasite isolates used could account for the differences noted with the Ugandan studies.

At least with respect to lake-related *S. mansoni* transmission, even though generally found to have low compatibility, *B. sudanica* is nonetheless considered more important than *B. choanomphala* since the former inhabits the lake shoreline and maintains large and visible populations, and as evidenced by two recent studies (Gouvras *et al*., 2017; Mutuku *et al*., 2019) most snails collected

from the lake with *S. mansoni* infections are of this taxon. However, given the higher experimental infection rates and higher rates of cercariae production noted here for *B. choanomphala*, it raises the possibility that this species is a more efficient transmitter of *S. mansoni* than *B. sudanica*, if it happens to be encountered by an *S. mansoni* miracidium in its deep-water habitat. It was observed that *B. choanomphala* potentially plays a significant, but under-appreciated role in the transmission of *S. mansoni* in Lake Victoria, a role also noted in the earliest studies of this taxon (Magendantz, 1972). Its role may not be fully appreciated because it is easy to underestimate its population size, this because dredging provides an inefficient and crude means of sampling its potential habitat areas. Alternative means of sampling like direct inspection of the bottom facilitated by scuba diving would be preferable but are rendered difficult because of the dangers posed by schistosomiasis transmission. An alternative sampling procedure that might provide a needed new perspective could be a catch, mark, release and re-catch sampling program.

The deep-water habitat of *B. choanomphala* can be viewed as a refugium in which the chances are lower for encountering miracidia of *S. mansoni* or of any other trematode whose eggs are deposited by definitive hosts living in or around the edges of the lake. At least in the case of *S. mansoni*, this effect might be partially offset by fishermen with the habit of defecating directly into deep water from their boats (Mutuku *et al*., 2019). Also, miracidia of trematodes infecting fish or turtles might be more likely to infect *B. choanomphala*. In addition to finding relatively few *B. choanomphala* infected with *S. mansoni* (Mutuku *et al*., 2019), they found infections of only 4 other trematode species in *B. choanomphala*, each of these in small numbers. By contrast, *B. sudanica* inhabiting the shoreline is colonized by at least 26 species of trematodes, including at higher overall prevalence. Persistent exposure to high burdens of a variety of trematodes could lead to selection for higher levels of innate resistance, consistent with what was observed for this species in its

interactions with *S. mansoni*. Lastly its noted that although *B. sudanica* and *B. choanomphala* are very similar with respect to typical genetic markers like 28S Nuclear rRNA and even mitochondrial genomes (Zhang *et al*., 2018), they do retain largely but not completely distinct habitat preferences and they can be differentiated by conchological features that are retained in culture. Furthermore, this study suggests they have measurable differences in compatibility with *S. mansoni*, a feature that may not be independent of the differences in their preferred habitats.

5.5: Conclusions

Using prevalence of infection following experimental exposure to 1, 5 or 10 miracidia of either sympatric or allopatric isolates of *S. mansoni*, length of prepatent period, and daily numbers of cercariae produced as measures of compatibility, Kenyan *B. choanomphala* proved to be more compatible with *S. mansoni* than *B. sudanica*. In comparison with earlier comparable studies, *B. pfeifferi* is more compatible with *S. mansoni* than either *B. choanomphala* or *B. sudanica*. The actual vectorial capacity of *B. choanomphala* is likely diminished by its preference for deeper water with ensuing lower probabilities for contact with *S. mansoni* miracidia or for any cercariae it might produce to reach human hosts. Nonetheless, the role *B. choanomphala* plays in *S. mansoni* transmission in the lake should not be ignored and currents or winds might actually lead to greater shoreline contamination with cercariae emanating from this taxon than generally imagined. Conversely, even though *B. sudanica* generally has low compatibility with *S. mansoni*, the fact that it occurs in vast numbers and it is an inhabitant of shoreline habitats increases the chances for some snails to become infected, and for the cercariae they produce to come into contact with humans. As borne out by recent studies reporting that most snails found infected with *S. mansoni* in the lake are *B. sudanica*, this snail in spite of its low compatibility still plays a major role in perpetuating

transmission. Its further noted that although *B. choanomphala* and *B. sudanica* are genetically very similar with respect to marker genes like cytochrome oxidase or even mitochondrial genome sequences, the differences noted in compatibility for these two species are noteworthy and suggest that individuals of *B. sudanica* are often refractory to infection, highlighting the need to reveal the underlying factors responsible for this lack of compatibility.

CHAPTER 6: SNAIL-RELATED DIFFERENCES IN RESPONSE OF *SCHISTOSOMA MANSONI* TO PRAZIQUANTEL TREATMENT AMONG RESPONDING AND PERSISTENT HOTSPOT VILLAGES ALONG THE KENYAN SHORE OF LAKE VICTORIA

6.1: Introduction

One of the most prevalent and persistent of the world's neglected tropical diseases is human schistosomiasis (Colley *et al*., 2014). There is currently considerable momentum to bring schistosomiasis under control and to proceed to elimination efforts (Rollinson *et al*., 2013; King, 2015; King and Dangerfield-Cha, 2008). An essential weapon in the elimination of schistosomiasis is the drug praziquantel (PZQ) which has been used extensively in a variety of control programs and has considerably reduced prevalence and intensity of infection (Webster *et al*., 2014). There is also a growing appreciation for the need for integrated control programs taking into account sanitation, provision of safe water, education, and acknowledging the fundamental role played by freshwater snails as vectors of the disease (Sokolow *et al*., 2016; Ross *et al*., 2017). The persistent success of schistosome parasites is owed substantially to their molluscan hosts which support the prolific production of human-infective cercariae, and that often exist in huge populations across a variety of freshwater habitats.

Africa harbors 90+% of the global burden of *S. mansoni* (Hotez and Kamath, 2009). In Kenya, there are three taxa of *Biomphalaria* snails that perpetuate transmission: 1) *B. pfeifferi*, whose distribution includes tributaries feeding Lake Victoria, and in small impoundments and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along the coast; 2) *B. sudanica*, mainly found along the shores of Lake Victoria and Lake Jipe and their

surrounding swamps; and 3) *B. choanomphala*, a deeper water inhabitant of Lake Victoria.(Brown, 1994; Dejong *et al*., 2001). The latter two taxa are likely members of the same species, and are frequently referred to as ecophenotypes or ectomorphs (Loker *et al*., 1993; Standley *et al*., 2011; Zhang *et al*., 2018).

Prevalence of intestinal schistosomiasis in Kenya is highest (>50%) in the Mwea irrigation scheme in central Kenya and in the Lake Victoria basin in western Kenya (http://www.thiswormyworld.org/maps/distribution-of-s-mansoni-survey-data-in-kenya). Recent studies assessing the prevalence of *S. mansoni* infection in school children in the lake basin reported overall prevalences of 60.5% and 69%, with villages on lake islands having 2-fold higher prevalence rates than mainland villages (Odiere *et al*., 2012; Samuels *et al*., 2012). Schools closer to the lake also had higher prevalence rates than those further from the lake (Odiere *et al*., 2012). Both higher prevalence's and likelihood of reinfection following treatment would logically be associated with more frequent contact with lake water containing infected snails (Wiegand *et al*., 2017).

Several chemotherapy-based initiatives have been undertaken to control schistosomiasis in the Lake Victoria region (Wiegand *et al*., 2017; Kabatereine *et al*., 2007; Crellen *et al*., 2016; Olsen *et al*., 2018; Kittur *et al*., 2017). Among them, the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project implemented school-based and village-wide mass drug administration (MDA) in varying treatment regimens over 4 consecutive years (2011-2015) for 150 Kenyan villages situated on or near the eastern shore of Lake Victoria (Wiegand *et al*., 2017). Villages were randomized with respect to six different treatment strategies (study arms) which involved combinations of school-based or community-wide treatments, with 3 study arms receiving annual treatment and 3 receiving treatment twice over a four-year period. Regardless of

study arm or age group of treated individuals, using spatial scan statistics a persistent hotspot area was identified in western Siaya County comprising west-facing villages along the open waters of the lake (Wiegand et al., 2017). Prevalence and intensity of infection did not decrease as much as it did in areas outside of the persistent hotspot area, the latter including villages along the more protected shores of Winam Gulf (Wiegand *et al*., 2017). Although this study could not clearly identify factors driving persistence of infection at hotspots, it reported an association between schistosome persistence and vegetation density (as measured by remote sensing) indicating a potential role for snail ecology.

Particular locations where the prevalence or intensity of schistosome infection does not fall to expected levels despite well-implemented, multiyear, MDA have been termed "persistent hotspots" or PHS, in contrast to responding villages (RESP) in which intensity and prevalence decline as expected. Why do some villages fail to respond to multi-annual treatment with PZQ? Several explanations can be offered including variability in efficacy of praziquantel among worm populations in different areas, levels of sanitation or availability of toilets, extent of drug coverage or compliance, availability of safe water supplies, extent of water contact, defecation behavior, or the extent to which they experience population movements such as resulting from fishing activities or proximity to ferry landings (Crellen *et al*., 2016; Magendantz, 1972; Kabatereine *et al*., 1996; Standley *et al*., 2010; Grimes *et al*., 2015).

Conditions associated with snail vectors may also vary considerably including the nature of the snail species present, their abundance and local compatibility with schistosomes, longevity of infected snails and the rate at which they produce cercariae, and their patterns of distribution relative to water contact sites and anthropogenic disturbance (Magendantz, 1972; Frandsen, 1979; Adriko *et al*., 2013; Lu *et al*., 2016: Mutuku *et al*., 2017; Standley *et al*., 2012; Lange *et al*., 2013).

Shoreline and bottom conditions can also play important roles in efficacy of transmission (Brown, 1994; Magendantz, 1972; Standley *et al*., 2010; Standley *et al*., 2012; Gouvras *et al*., 2017).

In addition to the shoreline-associated vegetation effects noted by Wiegand *et al*. as a possible factor associated with PHS, the dramatic presence and movements of large floating mats of the introduced water hyacinth, *Eichhornia crassipes*, are other factors in lake ecology that cannot be overlooked (Wiegand *et al*., 2017). Hyacinths have been suggested to provide a suitable environment for schistosome-susceptible snails and to facilitate their movements around the lake (Coles and Kabatereine, 2008). Plummer noted that significantly more *B. sudanica* were recovered from experimental enclosures containing hyacinths than control enclosures lacking them, and Ofulla *et al*. also concluded that *B. sudanica* was significantly associated with hyacinths in Nyanza Gulf (now referred to as Winam Gulf), Kenya (Plummer, 2005; Ofulla *et al*., 2010). Winam Gulf is one of the areas of the lake most affected by hyacinths, and four of the ten villages we investigated are found on the Gulf (Opande *et al*., 2004). Although hyacinth mats seem to favor snails in the short term, it has been noted that longer-term residence of hyacinth mats may be harmful to snails because of deficits in light penetration, water circulation, and oxygen availability, the latter due to decomposing hyacinths (Plummer, 2005).

In this study focus was drawn on the relationship of *Biomphalaria* snails to PZQ-based control efforts for *S. mansoni* in and around the Kenyan shoreline of Lake Victoria, one of the world's major hyper endemic foci of human schistosomiasis. During the two years immediately following the cessation of their MDA project, on four separate occasions, survey was done for *Biomphalaria* populations and their schistosome infection rates adjacent to 10 villages along the Kenyan shore of Lake Victoria with different degrees of responsiveness to treatment. In each of two collecting sites per village, general condition of the habitat was noted and searched for snails in shoreline-

associated and deeper water habitats in the lake, as well as in smaller habitats adjacent to the lake. Building on the results of Wiegand *et al*., investigation was done on the hypotheses that PHS and RESP villages differ with respect to the species of vector snails present, relative snail abundance, likelihood that the snails are infected with schistosomes or other trematodes, and numbers of cercariae in the water as measured by infection rates of sentinel mice. All of these factors could contribute to persistently high prevalence in the face of control operations.

6.2: Materials and Methods

6.2.1: Study sites

Wiegand *et al*. (2017) study already mentioned ten villages along the shores of Lake Victoria, western Kenya (Figure 6.1). The villages had annual school-based mass drug administration (MDA) for 4 consecutive years (Table 6.1). Following one of the definitions of Kittur *et al*., six of the villages (Minya, Agok, Migiro, Miyandhe, Kanyibok, Usenge) were persistent hotspots (PHS), locations where the absolute change in *S. mansoni* prevalence from the beginning of the control program to the end was ≤30% (Kittur *et al*., 2017). By contrast, for the four remaining villages (Kotieno, Seka Dok, St. Douglas Weta, Mumbo) recorded a drop in prevalence >30% and are considered responding (RESP) villages. In each village, two shoreline habitats were identified where there was evidence of human-water contact activities, and these were established as the sampling sites. Each of the 20 sampling sites was visited four times (April 2016, August 2016, January 2017 and May 2017).

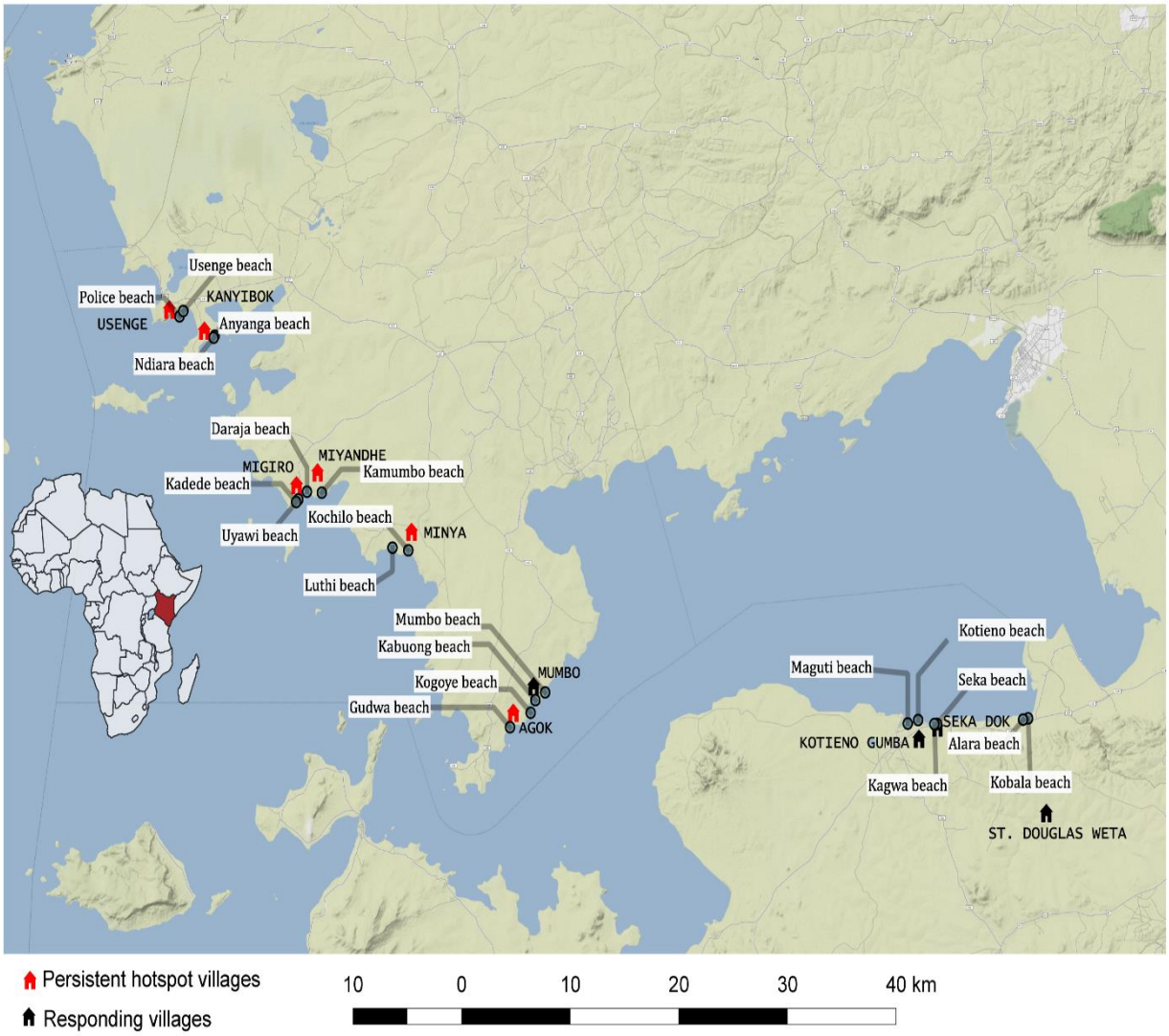


Figure 6.1: Map showing position of study villages along the shore of Lake Victoria, Kenya. The map was constructed using QGIS version 2.18.22 and 3.2.1, the basemap layer was added from the standard terrain tiles provided from http://maps.stamen.com through the OpenLayers function. The tiles are © Stamen Design, under a creative commons attribution (CC BY 3.0) license. The data for the locations were obtained manually using cellular phone GPS functionality according to the WGS1984 mercator projection.

Table 6.1: *S. mansoni* prevalence in schools within the study villages showing response to praziquantel treatment following four years of Mass Drug Administration. Data acquired from and shown with permission of the SCORE project. Sample sizes shown in parentheses.

School	Prevalence (%) Year 1 (2011)	Prevalence (%) Year 2 (2012)	Prevalence (%) Year 3 (2013)	Prevalence (%) Year 4 (2014)	Prevalence (%) Year 5 (2015)	Absolute Prevalence Change Year 1 Minus Year 5 as %
	Persistent Hotspot Villages					
MINYA	68.6 (n=97)	77(n=100)	75(n-97)	74(n-92)	82(n-97)	+13.4
AGOK	60.5(n-99)	59.8(n-93)	60.2(n-97)	67(n-86)	39(n-91)	-21.5
MIGIRO	69.4(100)	76.7(n-94)	49.2(n-87)	72(n-87)	48(n-88)	-21.4
MIYANDHE	78.6(86)	70.5(n-91)	71.4(n-87)	73.5(n-92)	54(n-94)	-24.6
KANYIBOK	91.8(100)	93(n-96)	83.7(n-98)	84(n-94)	83(n-100)	-8.8
USENGE	69.4(100)	68(n-100)	65(n-100)	46.5(n-100)	65(n-100)	-4.4
	Responding Villages					
KOTIENO	52.9(87)	40.9(n-92)	19(n-93)	16(n-84)	12(n-88)	-40.9
SEKA DOK	81.6(100)	73.2(n-97)	55.8(n-91)	48(n-90)	19(n-96)	-62.6
ST. DOUGLAS WETA	55.2(100)	27.4(n-94)	24(n-93)	19(n-87)	19(n-91)	-36.2
MUMBO	57.7(77)	30(n-74)	11.5(n-78)	15.6(n-79)	5.5(n-77)	-52.5

6.2.2 Snail Sampling

Two methods were used to collect snails from each of the 20 designated sampling sites: scooping from the shore and dredging from a boat as described in chapter 3. Shoreline habitats were typical for Lake Victoria consisting of emergent and submerged vegetation that was swept by scoops for snails (figure 6.2).

Figure 6.2: Snail collection at Anyang beach, Kanyibok by scooping method

Snails were also collected offshore by passing a dredge (0.75 m long and 0.4 m wide with attached sieve, 2 × 2 mm mesh size) along the bottom at each sampling site, dredge hauls covering a combined length of 150 meters were made, beginning at one meter depth and extending perpendicular to the shore to 10m depth (Figure 6.3). Live snails were picked from the dredged material. All snails were taken to the laboratory at KEMRI, Center for Global Health Research, Kisumu where they were sorted into species based on shell morphology characteristics, using standard taxonomic identification keys, counted and screened (Kristensen, 1987).

Figure 6.3: Snails collection by dredging method using a dredge tethered on a boat

6.2.3: Snails screening

Each individual snail was placed in an individual well of a 24-well plastic culture plate containing 1 ml of aged de-chlorinated tap water. The plate was placed in indirect sunlight for 2 hours between 10:00-13:00hr. Individual wells were then examined with the aid of a dissecting microscope for presence of cercariae. Using standard identification keys, cercariae were identified to basic taxonomic groups (Frandsen, 1984) Non-shedding snails were maintained in the laboratory for four weeks and screened again to enable detection of snails that harbored pre-patent infections at the time of initial collection (Mutuku *et al*., 2014). Additionally, samples of mammalian schistosome cercariae recovered from *B. sudanica* (each sample consisting of 4 cercariae) were extracted (QIAamp DNA Micro Kit, to elution volume of 45µl) and primers for the mitochondrial NADH dehydrogenase subunit 5 gene (*nad5*) used for amplification, following which bands were

visualized on a gel (302 bp expected band size for *S. mansoni* and 800 bp for the related schistosome *S. rodhaini*) as a check for the identity of the schistosome recovered (Lu *et al*., 2016).

6.2.4: Sentinel mice exposures

Mice were exposed to lake water at each near shore sampling site in floating cages that ensured the mice were exposed to a depth of no more than 5 mm as described in chapter 3. For each of the shoreline sampling sites, five cages each with 2 mice were tethered adjacent to locations where snails were collected (total of 20 mice per village per sampling time). Mice were in contact with the water for 3 hours (11:00 hr – 14:00 hr), an interval when shedding of cercariae by *S. mansoni*-infected snails is expected to be at its peak (Steinauer *et al*., 2008). Previous experience under lakeside conditions indicated that a three-hour period of exposure did not jeopardize survival of the mice, which was confirmed by the high survival rates of the mice retrieved. Mice from each sampling site were then marked with an identifying ear tag and maintained together until perfused at eight weeks post-exposure (Yolles *et al*., 1947). Recovered worms were counted and tabulated with respect to village, sampling site and sampling time.

6.2.5: Ethics statement

All experiments involving mice were approved by institutional animal care and use committees (IACUCs) at the Kenya Medical Research Institute (Protocol # KEMRI/ACUC/03.10.15) and at the University of New Mexico (Animal Welfare Assurance # D16-00565 (A4023-01, expiration 8/29/2021). All protocols and practices for the handling and manipulation of mice were in accordance with the guidelines of the American Veterinary Medical Association (AVMA) for humane treatment of laboratory animals.

6.2.6: Statistical analysis

To determine if PHS sites differed from RESP sites in snail-related factors, they were compared with respect to relative abundance of each snail (*B. sudanica* or *B. choanomphala*), the prevalence of infected snails, and the number of adult schistosome worms collected from the sentinel mice (as a measure of the force of infection). Collection sites were nested into village and were compared across the four time points. Because of the potential effects of large rafts of floating water hyacinths that temporarily occupy some sites, the presence or absence of hyacinths was included in the models.

6.2.6.1: Snail relative abundance

To determine whether relative abundance of either *B. sudanica* or *B. choanomphala* differed between PHS and RESP villages, between sites with hyacinths and no hyacinths, or over time, generalized linear mixed models in R using the package glmmTMB, with the random effect of village and using the family negative binomial 1 was performed (nbinom1) (Brooks *et al*., 2017).

Formula: glmmTMB (Abundance of either snail ~ Responder/Not + Hyacinth Y/N + timepoint + (1 | School),family=nbinom1(link=log))

6.2.6.2: Prevalence of infected snails

To determine whether the likelihood of detecting an infected snail was different in a RESP vs. PHS village, or in sites with and without hyacinths, a generalized linear mixed model using R was performed with the packages lmerTest and lme4 with the random effect of village and the family of binomial (logit link) (Bates *et al*., 2015). It also included the offset term of number of snails found, rescaled to be on the same scale as the binomial variables by dividing by 2 standard deviations (Gelman, 2008). This is because there is a strong positive correlation between the

likelihood of finding an infected snail and the total number of snails found (est=0.592+/-0.148, p=0.00005, statistics reported from the same model as described above but with total number of snails found included as a main effect rather than an offset). Data analyzed was only for *B. sudanica* because the number of infected *B. choanomphala* was prohibitively small.

Formula: glmer (smansoni. presence Y/N~ Responder/Not+ Hyacinth Y/N+ (1| timepoint) +(offset(rescaledtotalsudanicacount))+(1| School),family=binomial(link="logit"))

6.2.6.3: Sentinel mice

To evaluate whether infection prevalence in the sentinel mice differed between PHS and RESP villages, between sites with and without hyacinths and between time points, a generalized linear mixed model in R was performed using the package glmmTMB with a random effect of village, and family of negative binomial 1. This also included the term of total snails found at each site to account for snail relative abundance, however this variable was rescaled by dividing by 2 standard deviations to place it on the same scale as the binomial variables.

Formula: glmmTMB(total.number.of.schistosomes.in.mice ~ Responder/Not + Time + total.biomphalaria.snails + (1 | Village), family=nbinom1(link=”log))

Evaluation of whether the likelihood of finding a schistosome positive sentinel mouse differed between PHS and RESP villages, between sites with and without hyacinths and between time points was also done. The only difference between this model and the one described above is that the dependent variable is binomial (infected mouse found or not), rather than the burden within the infected mouse. For this, a model structure similar to the prevalence of infected snails was used. A generalized linear mixed model with family of binomial (link=logit) and the random effect of

village was performed. Once again accounting for the total number of snails collected was done, but rescaled it to be on the same scale as the binomial variables (Gelman, 2008).

Formula: glmer(smansoni.presenceinmice Y/N~ Responder/Not+ Hyacinth Y/N+ (1| timepoint)+(offset(rescaledtotalbiomphalariacount))+(1| Village),family=binomial(link="logit"))

6.2.6.4: Correlation of human prevalence and snail density

To determine if the abundance of snails at a particular site was associated with schistosome infection rates in humans, correlation of the total number of snails collected at a village (summed between collecting sites and all 4 time points) with both the initial and the final prevalence in humans at the corresponding village was done using Pearson's correlation in Graphpad Prism 7.0. Separate analyses for each snail species was done.

6.2.6.5: Biodiversity

To assess whether the likelihood of detecting a schistosome-infected *B. sudanica* at a site correlated with the presence of other digenetic trematodes in the snails, each of four commonly collected cercarial types were included (echinostome, strigeid, xiphidiocercariae, and amphistome) as a main effect in the same model as described above in the prevalence of infected snails.

Formula: glmer(smansoni.presence Y/N~ Responder/Not+ Hyacinth Y/N+amphistomes Y/N+xiphido Y/N, echinostomes Y/N, strigieds Y/N + (1| timepoint) + (offset(rescaledtotalsudanicacount)) + (1| village),family=binomial(link="logit"))

6.3: Results

6.3.1: Descriptive overview

A total of 12,156 snails (10,249 *B. sudanica* and 1,907 *B. choanomphala*) were collected from the study sites during four collection periods (Table 6.2, Figure 6.4). No *B. pfeifferi* were found in the lake habitats sampled. small streams or impoundments near the villages were searched and found them to be muddy, prone to drying and without *Biomphalaria* snails. At some times and collecting sites, hyacinth mats covered the water surface and were so dense as to preclude dredging (Figure 6.4). *B. sudanica* was recovered along the shoreline from all 20 sampling locations and *B. choanomphala* was dredged from deeper water at all 12 sampling locations in PHS villages whereas Mumbo was the only RESP village from which *B. choanomphala* was found (Table 6.2). Of note is that Mumbo is located closer to the PHS villages than the remaining three RESP villages (Figure 6.1). *B. choanomphala* was also sometimes found while scooping from shoreline collecting sites in three PHS locations, Kanyibok, Migiro and Usenge. At Usenge Beach, the shoreline *B. choanomphala* population persisted for at least five months on submerged vegetation along with another snail customarily recovered from deeper water, *Gabbiella humerosa*. Fifteen meters away, in a patch of emergent vegetation, *B. sudanica (*but not *B. choanomphala)* was found. *B. choanomphala* was also occasionally recovered from the shore intermingled with *B. sudanica*, as at the Anyanga beach sampling site at Kanyibok.

Hyacinth mats were noted during 3 of the collective totals of 48 total visits (6.25%) done to PHS sampling sites, and two of these times precluded dredging at such sites (Figure 6.4). Hyacinth mats were found at 11 of 32 collective visits (34.3%) at RESP sites, and at five of these times precluded dredging.

Table 6.2: The 20 sampling sites and their GPS coordinates, total number of *B. sudanica* and *B. choanomphala* collected from each, and the number of each species positive for *S. mansoni*.

Village	Habitat	Latitude	Longitude	***Biomphalaria sudanica***	**No. of snails (+) for *S. mansoni***	***Biomphalaria choanomphala***	No. of snails (+) for *S. mansoni*
Persistent Hotspot Villages							
	Kochilo beach	$00^0$14'10.96"S	$034^0$14'45.79"E	522	0	96	0
Minya	Luthi beach	$00^0$14'04.58"S	$034^0$13'58.74"E	758	1	282	0
	Gudwa beach	$00^0$21'26.52"S	$034^0$19'48.37"E	209	1	238	2
Agok	Kogoye beach	$00^0$20'51.94"S	$034^0$20'74.32"E	220	6	85	0
	Uyawi beach	$00^0$12'18.58"S	$034^0$09'18.36"E	728	1	89	0
Migiro	Kadede beach	$00^0$12'12.09"S	$034^0$09'11.74"E	132	0	100	0
	Kamumbo beach	$00^0$11'49.04"S	$034^0$10'28.17"E	48	0	15	0
Miyandhe	Daraja beach	$00^0$11'46.65"S	$034^0$09'44.11"E	103	0	71	0
	Anyanga beach	$00^0$05'22.49"S	$034^0$05'09.34"E	897	28	380	1
kanyibok	Ndiara beach	$00^0$05'27.33"S	$034^0$05'06.24"E	407	0	161	0
	Police beach	$00^0$04'34.80"S	$034^0$03'24.74"E	833	19	4	0
Usenge	Usenge beach	$00^0$04'21.49"S	$034^0$03'35.84"E	489	4	292	0
***B. sudanica* 60/5,346 (1.12%), *B. choanomphala* 3/1,813 (0.17%)**							
Responding villages							
	Kotieno beach	$00^0$21'09.07"S	$034^0$40'03.52"E	674	2	0	0
Kotieno	Maguti beach	$00^0$21'17.57"S	$034^0$39'32.50"E	649	16	0	0
	Seka beach	$00^0$21'18.58"S	$034^0$40'59.95"E	462	2	0	0
Seka Dok	Kagwa beach	$00^0$21'19"S	$034^0$40'52.12"E	834	1	0	0
St. Douglas Weta	Kobala beach	$00^0$21'05"S	$034^0$45'31.21"E	735	0	0	0
	Alara beach	$00^0$21'08"S	$034^0$45'16.01"E	584	0	0	0
	Kabuong beach	$0^0$20'20.06"S	$034^0$21'04.09"E	576	1	93	0
Mumbo	Mumbo beach	$0^0$20'0.99"S	$034^0$21'33.03"E	389	1	1	0
***B. sudanica* 23/4,903 (0.47%), *B. choanomphala* 0/94 (0%)**							

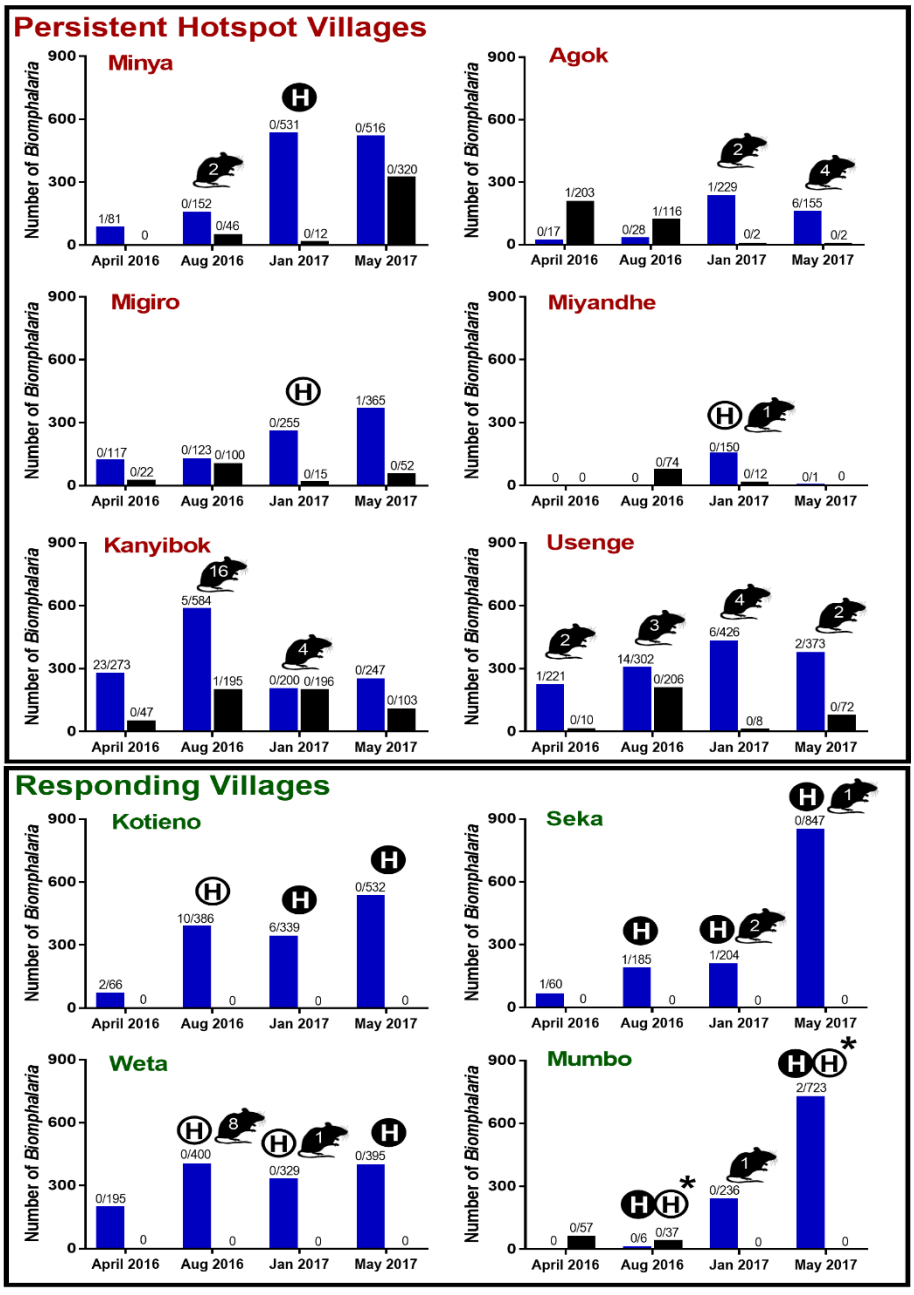

Persistent Hotspot Villages
Minya
Agok
Migiro
Miyandhe
Kanyibok
Usenge
Responding Villages
Kotieno
Seka
Weta
Mumbo
Number of Biomphalaria
April 2016
Aug 2016
Jan 2017
May 2017
Hyacinths, dredging done
Hyacinths, no dredging

Figure 6.4: Number of *B. sudanica* (blue bars) and *B. choanomphala* (black bars) collected at each of the four sampling times (April and August 2016, January and May 2017) from the 6 persistent hotspot villages on the top versus the 4 responding villages on the bottom. Snail numbers are summed between collection sites at a village. Numbers over bars indicate number of snails infected with *S. mansoni* shedders/total number of snails found. Numbers shown within mouse figures represent the number of worms recovered from sentinel mice at that time point (summed across mice and collection sites at that village). An H in a closed circle indicates hyacinths were present and dredging was done. An H in an open circle indicates hyacinths were present but dredging could not be done. An asterisk (*) indicates Kabuong Beach was dredged but not Mumbo Beach.

6.3.2: Snail relative abundance

Overall, far more *B. sudanica* than *B. choanomphala* were collected (Table 6.2, Figure 6.4). However, it should be noted that because the collecting methods differed for each snail taxon, these numbers are not directly comparable (e.g. there is not necessarily a larger population of *B. sudanica* than *B. choanomphala* in the lake). The relative abundance of snails was highly variable among sites and collection times. For instance, at Miyandhe, one of the PHS villages, with its rocky, wave-swept lakeshore habitat, few snails were recovered relative to all the other villages. It was not unusual for snails to increase or decrease several folds between collection time points at the same habitats (Figure 6.4).

In Lake Victoria, *B. sudanica* is considered a more important intermediate host of *S. mansoni than is B. choanomphala* because *B. sudanica* with its shallow water habitat use is more likely to contact miracidia from human feces deposited along the shore. Thus, it was predicted that snail driven differences between PHS and RESP sites would be reflected in this species. However, it was found

that PHS sites did not contain more *B. sudanica* snails than RESP sites when time point, village and hyacinths were taken into account (β = -0.0664, SE = 0.4603, P = 0.8854, Figure 6.5A). It was however noted that the three PHS villages (Minya, Kanyibok and Usenge) with the highest residual prevalence of *S. mansoni* are also the three villages with the highest overall counts of *Biomphalaria* snails. *B. sudanica* density was positively associated with the presence of hyacinths (β = 0.7704, SE = 0.2980, P = 0.0097, Figure 6.6) and collecting time point (β = 0.37912, SE = 0.09532, P < 0.0001).

In contrast to the *B. sudanica* results, *B. choanomphala* was more abundant at PHS sites (Figure 6.5B) compared to RESP sites (β = -2.1644, SE = 0.6975, P = 0.0091) and the presence of hyacinths and time point were not significantly associated with *B. choanomphala* density. Thus, results of the snail analysis indicated that only the deep-water taxon, *B. choanomphala*, differed between PHS and RESP sites. The other novel finding was that *B. sudanica* abundance was positively associated with the presence of hyacinths than when hyacinths were not present (P=0.00973).

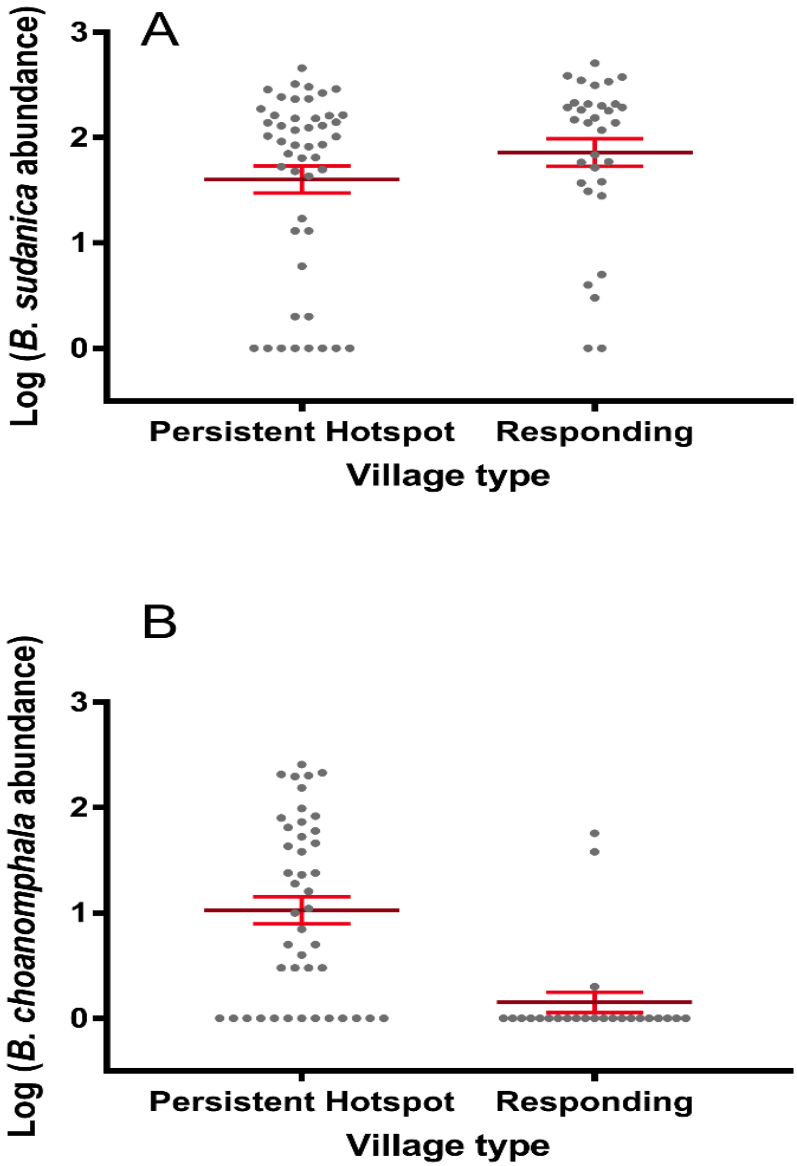


Figure 6.5: Relative abundance of *Biomphalaria* snails at persistent hotspot and responding sites of Lake Victoria in Kenya. Error bars represent standard error of the mean.

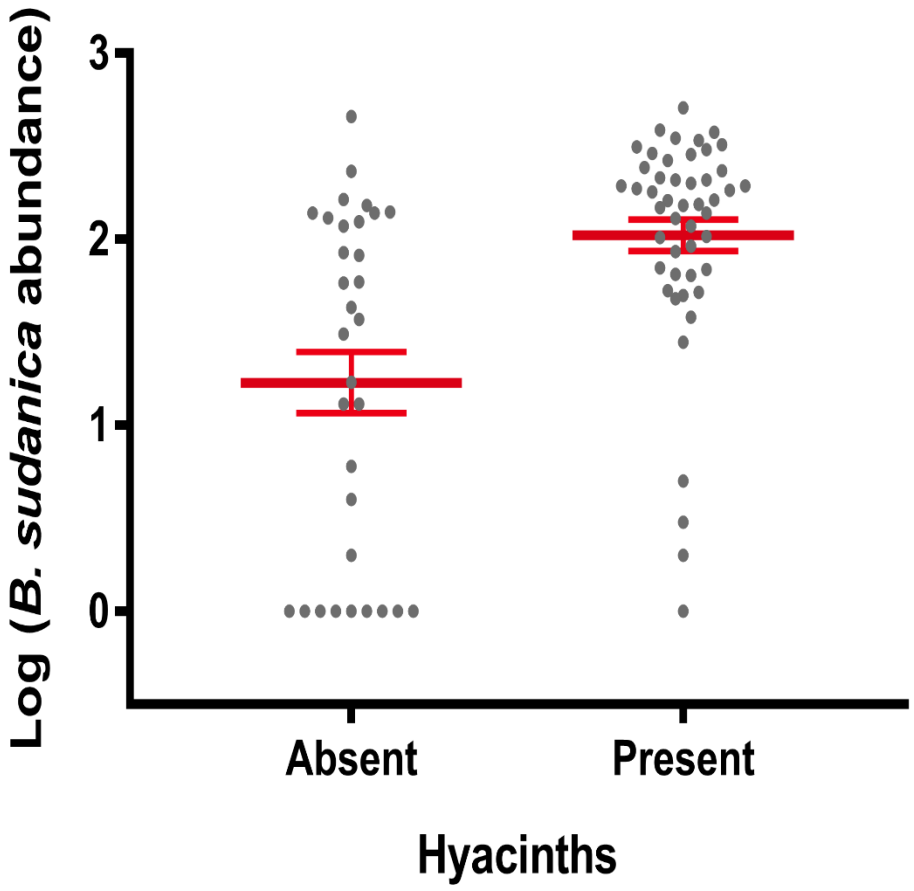


Figure 6.6. Association between number of *B. sudanica* collected and presence or absence of water hyacinths. Error bars represent standard error of the mean.

6.3.3: Prevalence of infected snails

The overall number of positive snails and prevalence for *S. mansoni* recovered among all 10 villages examined were higher for *B. sudanica* (83/10,249, 0.81%) than for *B. choanomphala* (3/1,907, 0.16%). Although few *B. choanomphala* were found positive for *S. mansoni*, all came from PHS villages. For *B. sudanica*, the total number of *S. mansoni*-infected snails and overall prevalence was higher in PHS villages (60/5,346, 1.12%) than from RESP villages (23/4,903, 0.47%). As a check on the identity of schistosome cercariae recovered, we amplified the *nad5* gene

from cercariae from 20 different positive snails, five from each of the time points, and representing several beach sites where schistosome cercariae were found (Lu *et al*., 2016). All 20 samples were verified as *S. mansoni* based on *nad5* band size, with one sample (Maguti Beach) exhibiting a double infection with both *S. mansoni* and *S. rodhaini*.

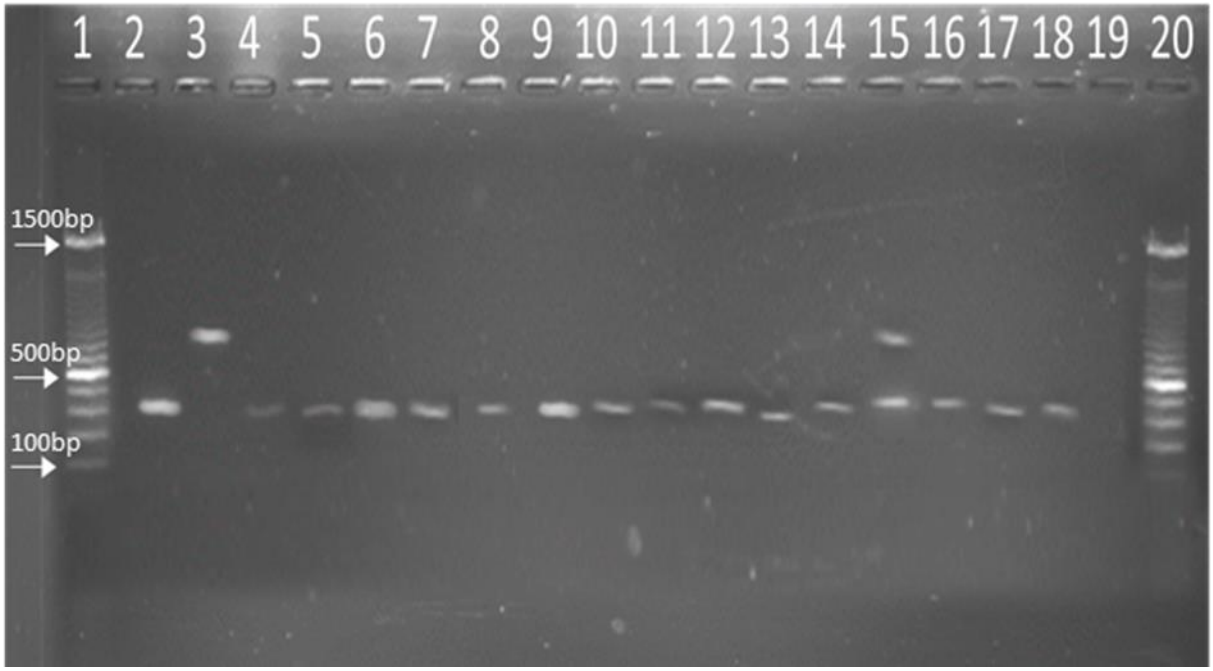


Figure 6.7: Gel photo showing band size for mammalian cercariae samples amplified for *nad5* gene, lane 1: GeneRuler 100 bp DNA ladder (Thermo Scientific, USA), lane 2: *S. mansoni* + ve control, Lane 3: *S. rodhaini* + ve control, lanes 4-18 show positive bands for *S. mansoni* except lane 15 which shows two bands, one for *S. rodhaini* and one for *S. mansoni*, lane 19: negative control were no sample was added; lane 20; GeneRuler 100 bp DNA ladder (Thermo Scientific, USA.

The distribution of *S. mansoni* infections among snails from the different villages is difficult to succinctly characterize. Two of the PHS villages with the highest residual post-treatment

prevalence's of *S. mansoni* in children, Kanyibok and Usenge, had the highest overall prevalence of positive snails (1.57% and 1.42% respectively (Table 6.2, Figure 6.4). From each locality though, most of these infections were found in a single collection raising the possibility they might have originated from one or a few human contamination events. Also, few shedders were found in Minya and Migiro, and none in Miyandhe, all PHS villages, whereas Kotieno, a RESP village, yielded the third most shedding snails, many of which were also found at a single time point. Ten of 24 sampling times (41.7%) from PHS villages yielded positive snails whereas 7 of 16 sampling times (43.8%) from RESP villages yielded snails positive for *S. mansoni*. Consequently, the number of *B. sudanica* infected with *S. mansoni* did not differ between PHS and RESP sites when time point, hyacinths, and village were all taken into account. There was a negative relationship that approached statistical significance between the number of infected snails collected and the presence of hyacinths at a site (β = -0.12362, SE = 0.07186, P = 0.0854), a point considered further in the discussion.

6.3.4: Sentinel mice

Worm recoveries from sentinel mice (Figure 6.4) were low (53 worms recovered, or 0.066 worms per mouse, or 0.022 worms/mouse/hour of exposure). The greatest number of worms acquired by a single mouse was 7. Of the total number of worms recovered, 40 (75.5%) were from PHS villages (0.083 worms/mouse) while 13 (24.5%) were from RESP villages (0.041 worms/mouse). Although the total number of adult schistosomes collected from mice caged in PHS sites was greater than those caged in RESP sites, there was no statistical difference between them when village, hyacinths, and time point were considered (Figure 6.8, β = -0.36479 SE = 0.5720, P = 0.5237). Examination of the viscera of positive sentinel mice revealed only the presence of *S. mansoni* eggs; no *S. rodhaini* eggs were found.

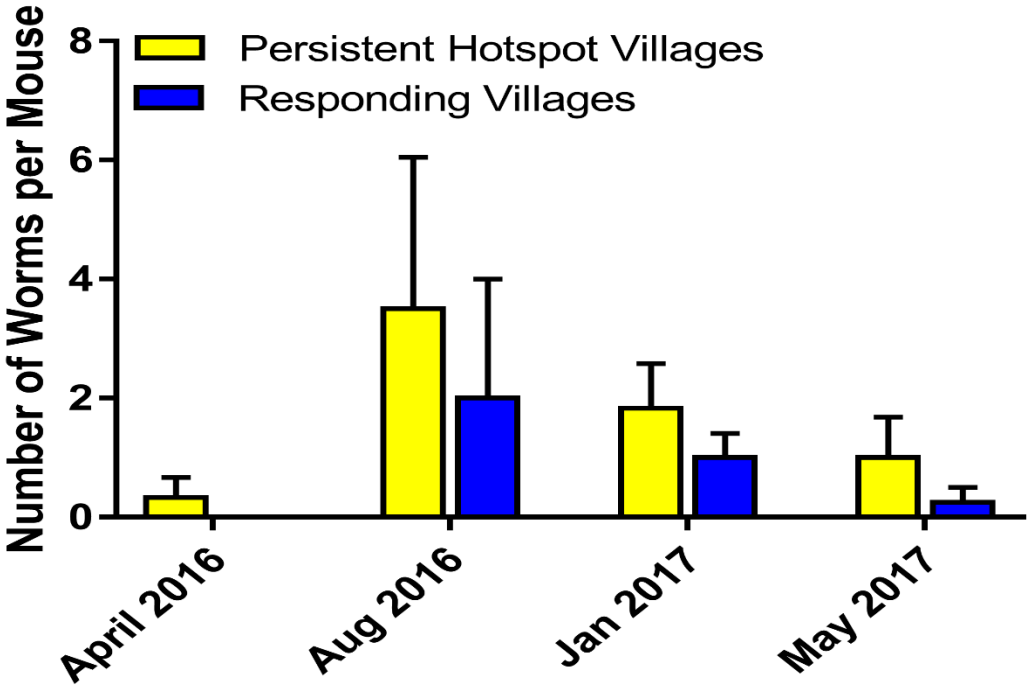


Figure 6.8: The total number of adult schistosomes collected from sentinel mice from persistent hotspot and Responding villages at the four time points.

6.3.5: Correlation between snail abundance and human prevalence

Total number of *B. sudanica* collected at a village was not significantly correlated with *S. mansoni* prevalence in humans (Figure 6.9) either before or after the drug treatment intervention (before: Pearson r = -0.0369, p = 0.460; after: Pearson r = -0.009, p = 0.49). However there was a strong correlation between the number of *B. choanomphala* collected at a village with *S. mansoni* prevalence in humans at the village (Figure 6.10) both before and after mass drug administration (before: Pearson r = 0.587, r^2 = 0.345, p = 0.037; after: Pearson r = 0.9034, r^2=0.816, p = 0.0002).

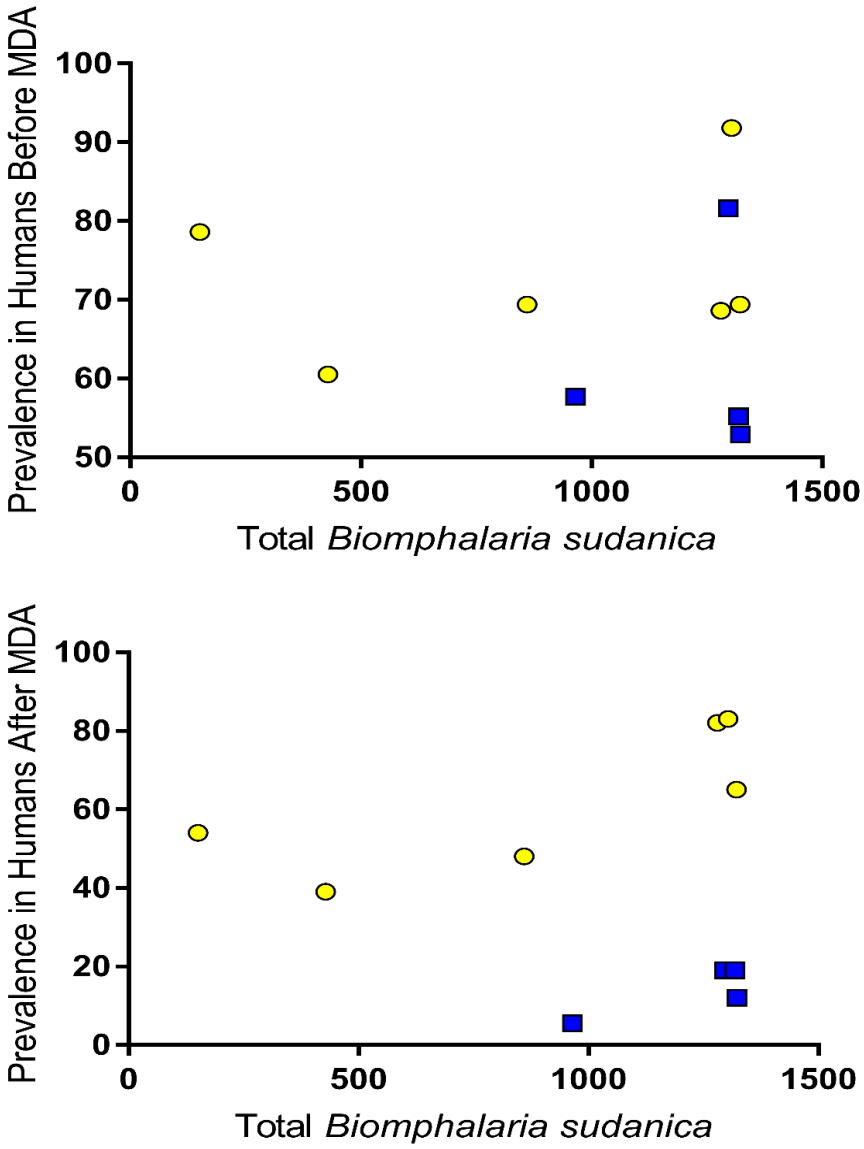


Figure 6.9: Correlation between total number of *B. sudanica* collected at each village and prevalence of schistosomiasis in humans before and after MDA. Yellow = persistent hotspot villages; Blue = responding villages.

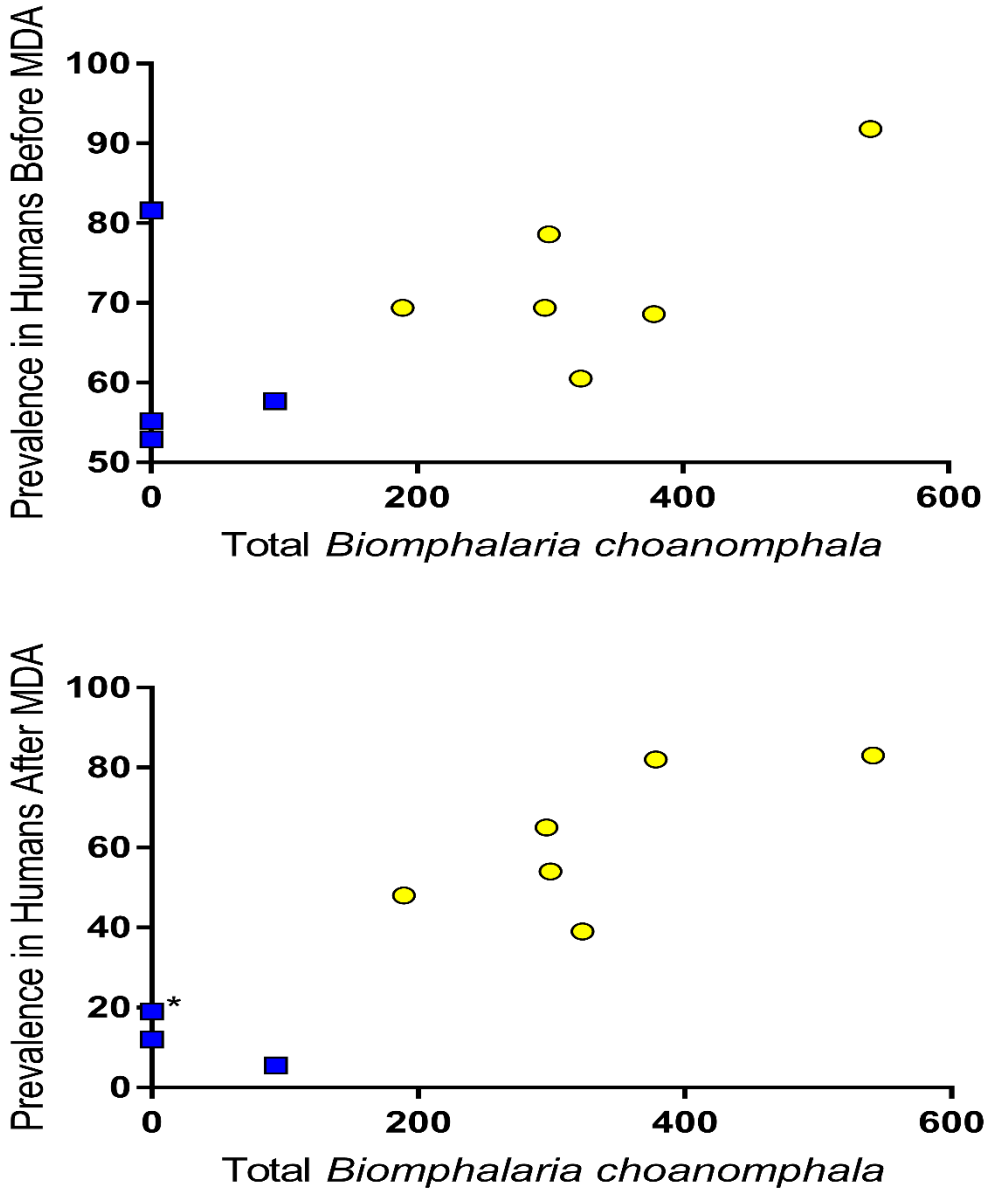


Figure 6.10: Correlation between total number of *B. choanomphala* collected at each village and prevalence of schistosomiasis in humans before and after MDA. Yellow = persistent hotspot villages; Blue = responding villages. Yellow = persistent hotspot villages; Blue = responding villages (* two villages with same prevalence).

6.3.6: Infection of *B. sudanica* with other digenetic trematodes

In all 10 villages, the number of trematode infections other than *S. mansoni* observed from *B. sudanica* (Table 6.3) exceeded those of *S. mansoni* (totals of 241 and 83, respectively). In PHS villages 60 of 184 trematode infections were of *S. mansoni* (32%), whereas in RESP villages, 23 of 150 (15%) were of *S. mansoni*. Overall prevalence of trematode infections including *S. mansoni* was similar between PHS and RESP villages (3.5 and 3.1%, respectively). Echinostomes were the most abundant non-*S. mansoni* infections with 112 snails infected from the ten study villages. No snails were observed to have double infections (i.e. observed to be simultaneously shedding two different kinds of cercariae). Although *S. mansoni* infections were consistently outnumbered by the aggregate totals of all other digenetic trematode infections, there were no statistical associations between the number of snails infected with *S. mansoni* and any of the other trematode species measured with the model employed.

With respect to having high overall total trematode prevalence, three villages stood out from the rest, the two PHS villages Kanyibok and Usenge and the one RESP village Kotieno (Table 6.3). All three villages had relatively high *Biomphalaria* populations, and yielded the most *S. mansoni* infections found in snails relative to other villages (Table 6.2).

Table 6.3: Number of *B. sudanica* snails collected from each village and number of snails infected with *S. mansoni* or other trematode cercariae. SM = *S. mansoni*, ECH = echinostomes, AMPH = amphistomes, STRIG = strigeids, XIPH = xiphidiocercariae, SM: ECH = ratio of *S. mansoni* to echinostomes.

VILLAGE	Total Snails	SM	ECH	AMPH	STRIG	XIPH	Total Number of Infected Snails	Trematode Prevalence
Persistent Hotspot Villages								
MINYA	1280	1	5	0	0	19	25	2.0
AGOK	429	7	4	0	1	7	19	4.4
MIGIRO	860	1	5	0	1	6	13	1.5
MIYANDHE	151	0	1	0	1	0	2	1.3
KANYIBOK	1304	28	22	7	4	1	62	4.8
USENGE	1322	23	10	6	12	14	65	4.9
TOTAL	5346	60	47	13	19	47	186	3.5
%		32	25	7	10	25		
Responding Villages								
KOTIENO	1323	18	29	7	17	2	73	5.5
SEKA DOK	1296	3	10	2	4	1	20	1.5
WETA	1319	0	13	1	6	15	35	2.7
MUMBO	965	2	13	1	2	4	22	2.3
TOTAL	4903	23	65	11	29	22	150	3.1
%		15	43	7	19	15		

6.4: Discussion

The SCORE-supported PZQ-based treatment program in the Lake Victoria region in western Kenya noted that PHS villages comprised approximately a third of the 150 study village. The village prevalence was determined by testing 9-12 year old students each year. These students had a relative risk of infection that was nearly 4 times higher than for students outside the PHS area (Wiegand *et al*., 2017). The SCORE program is by no means unique in identifying difficulties in reducing prevalence of infection in some villages adjacent to Lake Victoria (Crellen *et al*., 2016). The PHS for *S. mansoni* transmission identified by Wiegand *et al*., is in Bondo, Siaya County, western Kenya, and is associated with the west-facing villages adjacent to the open, unprotected waters of the lake. In contrast, the PZQ RESP villages faced the more protected waters of Winam Gulf (Wiegand *et al*., 2017). Among several factors postulated by Wiegand *et al*., 2017 to be associated with the PHS, and considered in this investigation were larger snail populations, or presence of different species of vector snails.

Of the three taxa of *Biomphalaria* commonly implicated in transmission of *S. mansoni* in Kenya, two were collected in this study: the shoreline-associated taxon *B. sudanica* and the deeper water taxon *B. choanomphala*. There was no obvious indication that *B. pfeifferi* played a role in *S. mansoni* transmission in any of the study villages, as no snails of this species were found in the lake, or in streams or small impoundments within the study localities. *B. sudanica* was found at all 20 collection sites and it was typically abundant as it is along much of the Lake Victoria shoreline (Brown, 1994: Loker *et al*., 1993; Magendantz, 1972; Gouvras *et al*., 2017), and approximately five times as many *B. sudanica* as *B. choanomphala* were recovered.

When taking into account all sampling sites, sampling times and presence of hyacinths, mean abundance of *B. sudanica* did not differ statistically between PHS and RESP villages. However, there was a significant trend for more *B. sudanica* to be present from the collecting sites if mats of hyacinths were present at the time of samples collection. Hyacinth mats were found in collection sites from both PHS and RESP villages but were more common in the RESP villages located along Winam Gulf, an area known to be prone to hyacinth intrusions (Kristensen, 1987). The recovery of *B. sudanica* from hyacinths and the association that was noted for them with hyacinths supports the assertion that floating hyacinth mats favour the dispersal of *B. sudanica* along the lakeshore (Coles and Kabatereine, 2008). Hyacinth movements are likely to have major effects on the population structure of *B. sudanica* in the lake.

By contrast, *B. choanomphala* was significantly more likely to be recovered from PHS than RESP villages. *B. choanomphala* was found in all 12 of the collecting sites in PHS villages and from only 2 of the 8 collecting sites from RESP villages, both from the village of Mumbo. Mumbo is located closer to the mouth of Winam Gulf than the remaining RESP villages which are located in more protected waters of the Gulf. Previous studies have also shown *B. choanomphala* to be rare or absent within Winam Gulf, possibly because the bottom substrate is too muddy, whereas, the substrate found off the shore of the PHS villages was a combination of sand and mud, known to be favourable to *B. choanomphala* (Loker *et al*., 1993; Magendantz *et al*., 1972). Gouvras *et al*., found *B. choanomphala* to be focally distributed in the Tanzanian waters of the lake, including in some more protected inlets (Gouvras *et al*., 2017). They noted, just as noted in this study, a general trend for this species to be more common in western than eastern locations, and considered a possible gradual change in substratum conditions from west to east might be responsible. Many other factors such as levels of dissolved oxygen, pollution and seasonal effects like rainfall may also influence

B. choanomphala populations (Lange *et al*., 2013; Gouvras *et al*., 2017). There was no association between the abundance of *B. choanomphala* and water hyacinths. Although hyacinth mats can cover areas of the lake where deeper water and hence *B. choanomphala* populations are present and could have adverse shading or other effects, the impression was that hyacinth mats along the more exposed shores away from the Gulf were more prone to be dispersed by winds.

With respect to the schistosome infections among the snails recovered, a couple of caveats should be kept in mind. One is that some infections are pre-patent at the time the snails are collected such that these infections would be missed. To overcome this, the approach here was simply to retain all non-shedding snails in lab aquaria and to re-shed them after four weeks. This is because there was an excellent snail-rearing facility near the field sites at in which mortality of snails during the four-week interval can be minimized. This approach has the advantage of detecting those infections that successfully transition to patency (cercariae producing infection). An alternative approach to detect pre-patent infections is to employ molecular xenomonitoring techniques to detect nascent infections at the time of collection (Standley *et al*., 2010; Lu *et al*., 2016; Gouvras *et al*., 2017; Abbasi *et al*., 2010). Such techniques can be very sensitive and specific, but are prone to error if the infection is very young at the time of assay, and some detected infections may have been doomed to fail, so transmission potential could potentially be overestimated (Lu *et al*., 2016). Also, extractions of individual snails for PCR-based or other types of molecular detection of pre-patent infections becomes prohibitively expensive and time-consuming when large numbers of snails need to be screened. Pooling of samples or use of eDNA based techniques may help but are likely to be accompanied by a loss of precision in estimates of snail infection rates (Sato *et al*., 2018). The second caveat is that the rodent-transmitted *Schistosoma rodhaini*, the sister species of S. *mansoni*, is known to be present in the Lake Victoria basin though it is rare by comparison with *S.*

mansoni (Steinauer *et al*., 2008). Extraction was done on mammalian schistosome cercariae (four cercariae per infected snail) from 20 different positive snails and all yielded *nad5* bands consistent with *S. mansoni*, with one of these samples indicating a co-infection with *S. rodhaini* (Lu *et al*., 2016). Molecular-based techniques unquestionably increase the accuracy of species identifications, but again pose some challenges in so far as an infected snail can produce thousands of cercariae over a period of several months and it is conceivable that the species composition of the schistosome cercariae could change during the interval. Again, with a large survey with many positive snails recovered, resources limit the ability to repeatedly sample individual cercariae produced by the same snail. Acknowledging this constraint, given the ubiquity of human infections in our study area, and that 20 out of 20 positive snails cercariae samples we tested were identified as *S. mansoni*, it is confident that the conclusions reached here pertain to *S. mansoni* infections.

With these caveats in mind, for snail infections of *S. mansoni*, both the number of positive snails found and prevalence were higher for *B. sudanica* than *B. choanomphala*. The overall prevalence of *S. mansoni* infections in all the *Biomphalaria* snails collected was almost 2-fold higher for the PHS villages than for the RESP villages, and the highest recovery of positive snails was found in Kanyibok (1.57%) and Usenge (1.42%), two of the villages with the highest residual prevalence of infections in school children. However, one RESP village Kotieno also showed a high overall prevalence of infected snails (1.36%). When all sampling sites, sampling times and presence of hyacinths were taken into account, PHS and RESP villages did not differ with respect to numbers or prevalence of infected *B. sudanica*. The few *B. choanomphala* found to be positive were recovered only from PHS villages.

There was also a suggestive negative relationship between hyacinth presence and infection rates of *B. sudanica* with *S. mansoni*. If a true association, this finding may reflect a decreased likelihood

of collecting infected snails when large hyacinth mats are present. Several explanations could account for this including higher mortality of infected snails due to oxygen depletion under hyacinth mats, reluctance of people to enter water for either defecation or washing purposes if hyacinths are present, or increased difficulty of miracidia in finding snails in spatially complex hyacinth mats. In general, the role of hyacinths in understanding the dynamics of schistosomiasis transmission in the lake requires further investigation.

Although fewer *B. choanomphala* than *B. sudanica* were collected, and their prevalence of infection with *S. mansoni* was relatively low, it would be incorrect to assume the former species has limited significance with respect to schistosome epidemiology in Lake Victoria. The dredge hauls across the extensive bottom surface of the lake can only be expected to retrieve a small proportion of the snails present and likely grossly under estimate the role of this snail species in perpetuating transmission. At least two factors might facilitate infections in *B. choanomphala*: currents moving from offshore to deeper water, and fishermen who may defecate directly into deeper water from their boats. In this regard, failure to include fishermen in control programs may favour *B. choanomphala* mediated transmission. Another factor potentially favouring involvement of *B. choanomphala* in transmission is that experimental infection studies of Kenyan derived specimens suggest this species, like *B. pfeifferi*, is inherently more susceptible to *S. mansoni* than *B. sudanica* (Mutuku *et al*., 2017; Mutuku *et al*., 2014). Gouvras *et al*., working in southern Lake Victoria found more *B. choanomphala* positive for *S. mansoni* than found in this study: 12.2% of all *S. mansoni* shedders were *B. choanomphala* in their study vs. only 3.5% in this study (Gouvras *et al*., 2017). In both studies though, *B. sudanica* was identified as the major vector for *S. mansoni* in the lake.

Considering the second measure of the force of transmission examined, namely sentinel mice infections, there was no significant difference in mean number of worms recovered between PHS and RESP villages, though worm recoveries were approximately twice as high in the former and a positive correlation was noted across villages between the total number of snails shedding *S. mansoni* and the number of worms recovered from mice. Results of snail collections and worm recoveries were concordant in 60% of the 40 sampling times, but presence of *S. mansoni* shedding snails by no means guaranteed the recovery of worms from sentinel mice placed nearby, and vice versa: recovery of worms from sentinel mice was frequently noted at sampling locations, and at times no shedding snails were found. This suggests that neither method perfectly reflects the possibility of transmission at a particular locality at a certain time.

Not unexpectedly, and as reported from previous studies, *Biomphalaria* snails shedding other trematode cercariae were also frequently collected (Rowel *et al*., 2015; Okeke and Ubachukwu, 2017; Mohammed *et al*., 2016; King *et al*., 2009). Though non-schistosome infections exceeded *S. mansoni* infections by a factor of 2.9, there was no significant positive or negative associations with any of the groups of trematodes with *S. mansoni* infections. The three villages that yielded the most *S. mansoni* snail infections (PHS villages Kanyibok and Usenge and RESP Kotieno) also yielded by far the most infections with other trematodes, suggesting they are general "trematode-transmitting hotspots". One possibility to explain this is that the overall susceptibility to trematode infection is higher in snails from collecting sites in some villages than others. This possibility is currently being examined with respect to susceptibility to *S. mansoni*. Another explanation is that certain water contact points along the lakeshore attract intense activity by a variety of potential definitive hosts including people, domestic animals like cattle or goats and wild birds, the latter

often thronging to locations where fishermen bring their catches. People often contact water extensively at such sites, making them potentially dangerous potential transmission sites.

In comparison to *B. sudanica*, *B. choanomphala* had fewer *S. mansoni* infections and fewer infections with trematodes of other species (only 4 other species noted). Deep water has been considered a refugium, or in the parlance of King *et al*., a "co-evolutionary cold spot" that limits exposure of snails to digenetic trematodes (King *et al*., 2009). In comparison, shoreline habitats are visited by numerous definitive hosts and can be considered as "co-evolutionary hot spots" for digenetic trematode infections, supported by the many trematode species noted to be transmitted by *B. sudanica* (King *et al*., 2009).

This study poses several questions requiring clarification. For instance, it might be argued that PHS villages have higher prevalence of human infections because the snails adjacent to such villages are more susceptible to *S. mansoni* infection. Also, in need of additional clarification is the extent to which the gene pools of *B. sudanica* and *B. choanomphala* co-mingle. Studies of mitochondrial and nuclear markers indicate the two taxa are not highly divergent genetically and should probably be considered as a single species with the species name with precedence yet to be decided (Standley *et al*., 2011; Zhang *et al*., 2018). It is clear though that pockets of snails with the characteristic *B. choanomphala* phenotype can be recovered from the shoreline over a period of several months. The extent to which snails of the two phenotypes interbreed, the appearance of the progeny, and the nature of genes and alleles dictating compatibility to schistosomes and other trematodes all remain to be investigated.

6.5: Conclusions

Results of this study did not find significant differences between PHS and RESP villages for *B. sudanica* with respect to relative abundance or numbers of snails shedding *S. mansoni* cercariae, or in the number of worms recovered from sentinel mice. Although *B. sudanica* was present in all the villages studied and based on the number of positive snails of this species recovered it is the major vector for *S. mansoni* in all villages, *B. choanomphala* was found in all PHS villages and in only one RESP village. The relative abundance of *B. choanomphala* was significantly higher in PHS villages. *B. choanomphala* may provide an under-appreciated and more diffuse and difficult to measure alternative route of transmission along much of the shore of the lake. Many other trematode species infect *Biomphalaria* snails in Lake Victoria but their overall prevalence is low and suggests they do not have a dramatic primary effect on *S. mansoni* prevalence rates in snails. Evidence of active *S. mansoni* transmission was found in all 10 villages investigated, not surprising from the standpoint that the lake supports massive populations of *Biomphalaria* snails and sanitary facilities in rural areas around the lake are scarce. However, distinct differential responsiveness to annual MDA with PZQ was somewhat surprising. Nevertheless, opportunities for transmission and reinfections are very likely to continue to occur, even in RESP villages. Control of *S. mansoni* in and around the lake will remain a daunting challenge and its persistence argues more persuasively than ever for implementation of improved sanitation and provision of safe water supplies as important parts of an integrated and sustained three-country basin-wide control approach, and as fundamental rights for the people living in the area.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1: Discussion

In this study the vectorial competence of the local *Biomphalaria spp* for *S. mansoni* in Kenya were examined using several parameters namely parasite infection rates (proportion of schistosome-exposed snails that become infected and produce (shed) cercariae, infection pre-patent period (time required to complete sporocyst development and first release of cercariae following exposure to miracidia, longevity of infected snails, duration of actual shedding of cercariae by the schistosome-exposed snails, and daily cercarial output of infected snails. The study also examined snail-related factors that may explain persistence of *S. mansoni* transmission in PHS villages (which had >30 *S. mansoni* prevalence after a 4-year PZQ treatment campaign) compared with the responding villages (which had <30% infection prevalence after the 4-year PZQ treatment campaign) associated with Lake Victoria, western Kenya. For this study 10 villages were selected for investigation, comprising 6 PHS villages located along the west-facing shore of Lake Victoria, and 4 RESP villages located on the Winam Gulf of Lake Victoria.

Experimental exposures of field-collected *B. pfeifferi* to single miracidium infections consistently attained 40% infection rates and above, regardless of whether the *S. mansoni* parasite was derived from locations sympatric to the *B. pfeifferi* population or from an allopatric location. In this experiment, 80% of the juvenile *B. pfeifferi* from Mwea (in central Kenya) became infected after exposure to single *S. mansoni* miracidium isolated from naturally infected individuals from Nawa on the Lake Victoria shore, a site approximately 300kms away from Mwea. These results are in agreement with other studies (Frandsen, 1979; Southgate *et al*., 2000; Ibikounlé *et al.*, 2012; Lu *et al*., 2016), confirming a high level of compatibility of *S. mansoni* with both sympatric and allopatric *B. pfeifferi*, including those in which the snails and schistosomes used originated from other

continents (Frandsen, 1979; Ibikounlé *et al*., 2012). In contrast, *S. mansoni* compatibility with *B. sudanica* was consistently low in both sympatric and allopatric combinations, again in agreement with the results obtained by Frandsen (1979) for studies involving the same species. The results of the present study, however, contrasted sharply with the results obtained by Adriko *et al*., (2013) who demonstrated that *B. sudanica* was more susceptible than *B. pfeifferi*. The Nawa *S. mansoni* isolate used in the present study (which was recovered from individuals living near the shore of Lake Victoria and most likely originated from *B. sudanica*), never infected more than 25% of sympatric *B. sudanica*, even though this same isolate proved to be very compatible with allopatric *B. pfeifferi* from Mwea (with 80% infection rates).

In a separate experiment, when laboratory raised F1 generation of *B. choanomphala* and *B. sudanica* were exposed to varying doses of field derived sympatric or allopatric *S. mansoni* cercariae, *B. sudanica* again consistently attained low infection rates of less than 20%, whether the *S. mansoni* isolate was derived from locations sympatric to the snail host or from an allopatric location, with a 92% lower odds of being infected, compared with *B. choanomphala*, after accounting for both miracidia dose and source (GLM, β = 0.08, z = -12.05, P = <0.0001). These results were similar to results of other studies, which also, showed that *B. sudanica* consistently attained low infection rates (Lu *et al*., 2016; Mutuku *et al*., 2017).

With respect to miracidia dose, it was noted that for *B. choanomphala*, there was a steady increase in infection rates when the cercariae dose was increased from 1 to 5 or 10 miracidia per snail, with the odds of becoming infected with 5 or 10 miracidia being 612 % and 531 % higher than the odds of being infected with 1 miracidia (GLM, Dose 5: β = 7.12, z = 5.19, P = <0.0001; Dose 10: β =6.31, z = 4.93, P = <0.0001). This could imply that in localities where transmission is perpetuated by *B. choanomphala*, increased seeding of the habitat with more fecal matter from infected

individuals will result in an increase in the number of infected snails, hence an increase in force of transmission.

With regard to the number of cercariae released per day, on average field derived *B. pfeifferi* produced more cercariae compared with *B. sudanica*. For individual *B. pfeifferi*, >2,000 cercariae were often recovered from a 2-hr shedding period, by contrast, none of the experimentally exposed *B. sudanica* were observed to shed more than 900 cercariae in a comparable interval, in agreement with the results of Frandsen (1979). Following exposure of laboratory raised F1 generation snails, the average number of cercariae produced by *B. choanomphala* was generally higher than that produced by *B. sudanica* with the expected number of cercariae shed by *B. sudanica* being 46 % lower than for *B. choanomphala*, after accounting for parasite dose and source (GLM, $\beta = 0.54$, $z = -4.34$, $P = <0.0001$. A key determinant of force of transmission is the number of cercariae produced, and the more the cercariae in the water, the greater the chances of some of the cercariae coming into contact with humans and penetrating intact skin, and thereby, enter into the blood circulation and initiate infection.

In an attempt to identify snail-related factors that could account for the differences in *S. mansoni* prevalence between the 6 PHS villages and the 4 RESP villages investigated in the present study, it was observed that *B. sudanica* and *B. choanomphala*, both commonly implicated in the transmission of *S. mansoni* in Lake Victoria, in western Kenya, were regularly collected from collection sites in the lake. Whereas, *B. sudanica* was found in all the 20 collection sites in both PHS and RESP villages, *B. choanomphala* was found in all the 12 collection sites in PHS villages, but only from 2 of the 8 collection sites from RESP villages. Also, the prevalence of *S. mansoni* infections among the B. sudanica was higher than for *B. choanomphala*. Interestingly, the overall prevalence of *S. mansoni* infections in all the *Biomphalaria* snails collected was nearly 2-fold

higher for the PHS villages than for the RESP villages, and the highest recovery of positive snails was found in Kanyibok (1.57%) and Usenge (1.42%), two of the villages with the highest residual prevalence of infections in school children, suggesting that the force of Sm transmission was most likely greater in PHS villages than in RESP villages. Although fewer *B. choanomphala* than *B. sudanica* were collected, and prevalence of *S. mansoni* infection in these snails was relatively low, it would be incorrect to assume that the *B. choanomphala* has limited significance with respect to schistosome epidemiology in Lake Victoria. The dredge hauls across the extensive bottom surface of the lake can only be expected to retrieve a small proportion of the snails present and likely grossly under estimate the role of this snail species in perpetuating transmission.

When the second measure of the force of transmission examined (namely sentinel mice infections) was considered, there was no significant difference in mean number of worms recovered between PHS and RESP villages. Nevertheless, worm recoveries were approximately twice as high in the PHS villages, and a positive correlation was noted across villages between the total number of snails shedding *S. mansoni* and the number of worms recovered from sentinel mice. However, the presence of *S. mansoni* shedding snails by no means guaranteed the recovery of worms from sentinel mice placed nearby, and vice versa.

7.2: Conclusions

The results of the present study suggest that while field collected *B. pfeifferi and B. sudanica* can support full development of either allopatrically or sympatrically derived *S. mansoni*, regardless of snail size or age, *B. pfeifferi* is significantly more likely to become infected with *S. mansoni* and has higher daily rates of cercariae output than with *B. sudanica.* In other words, *B. pfeifferi* is more compatible with the local *S. mansoni* than *B. sudanica*, and is likely to be a more efficient

transmitter of *S. mansoni* than *B. sudanica*. Given that the present study also, observed that a proportion of *B. pfeifferi* shedders survived for several months and continued to shed S. *mansoni* cercariae, an indication that such snails if present in transmission sites could continue transmitting *S. mansoni* infection, and could play a significant role in initiating re-infections in the face of a sustained mass drug administration.

In Lake Victoria, western Kenya, where both *B. choanomphala* and *B. sudanica* are implicated in the transmission of *S. mansoni*, there was a significant difference in infection rates between the 2 snail species in experimental exposures, with *B. choanomphala* being more compatible with sympatric *S. mansoni* than with the allopatric parasite, but *B. sudanica* had consistently low rates of infection regardless of the parasite source. Besides, *B. sudanica* produced marginally lower number of cercariae relative to *B. choanomphala*. Although the deep water dwelling *B. choanomphala* had higher infection rates and produced more cercariae than *B. sudanica*, its vectorial competence could be curtailed by the fact that the cercariae it produces encounters large volumes of water and water currents, hence the chances of them swimming to the shoreline where they can get a chance to come into contact with humans, is significantly reduced. Even though *B. sudanica* seems less efficient in transmission of the parasite on a per snail basis, it occurs in vast numbers, thus compensates for its low compatibility, as such it can readily sustain transmission on the lake shore.

With respect to differences in response to PZQ treatment between the PHS and RESP villages, evidence of active *S. mansoni* transmission was found in all the 10 villages investigated, which is not surprising because the lake supports massive populations of *Biomphalaria* snails and sanitary facilities in villages around the lake are scarce. Apparently, *B. sudanica* was present in all the snail collection sites in the 10 villages studied, and based on the number of positive snails of this species

recovered it seems to be the major transmitter of *S. mansoni* in Lake Victoria, western Kenya. However, given the presence of *B. choanomphala* in all the PHS villages, and only in 1 RESP village located adjacent to the open western facing shoreline where the PHS villages are located could imply that *B. choanomphala* may be providing an under-appreciated alternative route of transmission in the lake.

7.3: Recommendations

Several questions arising from the present study may require further research as enlisted below.

1) Further studies are needed to determine if the differences in the breeding systems of *B. pfeifferi* (preferential selfer) and *B. sudanica* (out crosser) could be instructive in susceptibility of the 2 snail species to *S. mansoni*. Does interaction of *S. mansoni* with a sexually reproducing host confer on it properties of infectivity that guarantee it a higher likelihood of success when confronted with a selfing species like *B. pfeifferi?*.
2) This study has shown that both *B. pfeifferi* and *B. choanomphala* are generally very susceptible to *S. mansoni* regardless of its origin, and even though *B. sudanica* seems less susceptible to the parasite, it occurs in vast numbers on the shores of Lake Victoria, hence control efforts should be directed to all locations where there is presence of *Biomphalaria* snails regardless of the species because all the 3 taxa can sustain transmission and reinfections after chemotherapy.
3) Evidence of factors favoring active transmission of *S. mansoni* in all villages studied indicates that schistosomiasis around the lake will remain a public health concern hence the need for implementation of community-wide chemotherapy, improved sanitation and provision of safe water supplies as parts of an integrated control approach.

REFERENCES

Abbasi, I., King, C. H., Muchiri, E. M., and Hamburger, J. (2010) Detection of *Schistosoma mansoni* and *Schistosoma haematobium* DNA by Loop-Mediated Isothermal Amplification: Identification of Infected Snails from Early Prepatency. *American journal of Tropical Medicine and Hygiene* **83** (2):427-432.

Adriko, M., Standley, C. J., Tinkitina, B., Mwesigwa, G., Kristensen, T. K., Stothard, J. R., and Kabatereine, N. B. (2013) Compatibility of Ugandan *Schistosoma mansoni* isolates with *Biomphalaria* snail species from Lake Albert and Lake Victoria. *Acta Tropica* **128** (2): 303-308.

Agola, L. E., Steinauer, M. L., Mburu, D. N., Mungai, B. N., Mwangi, I. N., Magoma, G. N., Loker E.S., and Mkoji, G. M. (2009) Genetic diversity and population structure of *Schistosoma mansoni* within human infra populations in Mwea, central Kenya assessed by microsatellite markers. *Acta Tropica* 111 (3):219-25.

Agola, L. E., Mburu, D. N., DeJong, R. J., Mungai, B. N., Muluvi, G. M., Njagi, Loker, E.S, and Mkoji, G. M. (2006) Microsatellite typing reveals strong genetic structure of *Schistosoma mansoni* from localities in Kenya. *Infection, Genetics, Evolution* **6** (6):484-490.

Ana, A., Maurício, B., Matildes, P., Mitermayer, R., Isabel, P., and Ronald, B. (1998) *Schistosoma mansoni* infection and nutritional status in schoolchildren, a randomized, double-blind trial in northeastern Brazil. *American Journal of Clinical Nutrition* **68** (6): 1247-1253.

Anderson, R. M., and May, R. M. (1979) Prevalence of schistosome infections within molluscan populations, observed patterns and theoretical predictions. *Parasitology* **79**:63.

Bates, D., Maechler M., Bolker B., and Walker S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**:1-48.

Brooks M. E., Kristensen K., Van Benthem K. J., Magnusson A., Berg C. W., Nielsen A., Skaug H. J., Maechler M., and Bolker B. M. (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* **9** (2):378-400.

Brown D. S. 1994. Freshwater snails of Africa and their medical importance, 2nd edition. CRC Press, Boca Raton, Florida, 608 p.

Brown D. S. (2005. Freshwater snails of Africa and their medical importance.2nd ed. USA. Taylor and Francis Limited.

Caldas I. R., Campi-Azevedo A. C., Oliveira L. F., Silveira A. M., Oliveira R. C., and Gazzinelli, G. (2008) Human *Schistosoma mansoni*, immune responses during acute and chronic phases of the infection. *Acta Tropica* **108** (23):109-117.

Campbell G., L. R. Noble D. Rollinson, V. R. Southgate, J. P. Webster, and C. S. Jones. (2010) Low genetic diversity in a snail intermediate host (*Biomphalaria pfeifferi* Krauss, 1848) and schistosomiasis transmission in the Senegal River Basin. *Molecular Ecology* **19** (2): 241-256.

Centers for Disease Control and Prevention. (2009) Schistosomiasis. Retrieved from. http, //www.dpd.cdc.gov/ dpdx/images/ParasiteImages/ Schisto Schistosomiasis /Schistomes_LifeCycle

Centers for Disease Control and prevention.2012. Schistosomiasis. Retrieved from. http, //wwwnc.cdc.gov/travel/yellowbook/ 2012/chapter-3-infectious-diseases-related-to-travel/schistosomiasis.htm.

Charbonnel N., Rasatavonjizay R., Sellin E., Brémond P., and Jarne P. (2005) The influence of genetic factors and population dynamics on the mating system of the hermaphroditic freshwater snail. *Biomphalaria pfeifferi*. *Oikos* **108** (2):283-296.

Chandiwana S. K. (1987) Technique to detect schistosome cercariae in natural water through exposure of sentinel hamsters. *The Journal of Parasitology* Vol. 73 (2): 452-454.

Chitsulo L., Engels D., Montressor A., and Savioli L. (2000) The global status of schistosomiasis and its control. *Acta Tropica* **77**:41–51.

Coles G. C. and Kabatereine N. B. (2008) Water hyacinth and the transmission of schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102** (6):619-620.

Colley D. G., Bustinduy A. L., Secor W. E., and King C. H. (2014) Human schistosomiasis. *Lancet* **383** (9936):2253-2264.

Coulibaly G., and Madsen H. (1990). Seasonal density fluctuations of intermediate hosts of schistosomes in two streams in Bamako, Mali. *Journal of African Zoology* **104**: 201-12.

Crompton T. (1999) How much human helminthiases is there in the world?. *Journal of Parasitology* **85**: 397.

Davies C. M., Webster J. P., and Woolhous M. E. (2001) Trade-offs in the evolution of virulence in an indirectly transmitted macroparasite. *Proceedings of the Royal Society of London: Biological Sciences* **268** (1464):251-257.

DBL-WHO. 1998. A field guide to African Fresh water snails. Charlottenlund, Denmark: Geneva: WHO.

De Barro P. J., Murphy B., Jansen, C.C., and J. Murray J. (2011) The proposed release of the yellow fever mosquito, *Aedes aegypti* containing a naturally occurring strain of *Wolbachia pipientis*, a question of regulatory responsibility. *Journal Verbrauch Lebensm* **6**:33-40.

DeJong R. J., J. A. Morgan W. L., Paraense J. P., Pointier M., Amarista P. F., Ayeh-Kumi A. Babiker C. S., Barbosa P., Brémond A., Pedro Canese A., de Souza C. P., Dominguez C., File S., Gutierrez A., Incani R. N., Kawano T., Kazibwe F., Kpikpi J., Lwambo N. J., Mimpfoundi R., Njiokou F., Noël Poda J., Sene M., Velásquez L. E., Yong M., Adema C. M., Hofkin B. V., Mkoji G. M., and Loker E. S. (2001) Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with implications regarding its role as host of the human bloodfluke, *Schistosoma mansoni*. *Molecular Ecology and Evolution* **18** (12):2225-2239.

Dejong R. J., Morgan J. A., Wilson W. D., Al-Jaser M. H., Appleton C. C., Coulibaly G. , D'Andrea P. S., Doenhoff M. J., Haas W., Idris M. A., Magalhães L. A., Moné H., Mouahid G., Mubila L., Pointier J.-P., Webster J. P., Zanotti-Magalhães E. M., Paraense W. L., Mkoji G. M., and Loker E. S. (2003) Phylogeography of *Biomphalaria glabrata* and *B. pfeifferi*, important intermediate hosts of *Schistosoma mansoni* in the New and Old World tropics. *Molecular Ecology* **12** (11):3041-56.

Ebert D. (1994) Virulence and local adaptation of a horizontally transmitted parasite. *Science Journal* **265** (1):1084-1086.

Esch G. W., and Fernandez J. C. (1994) Snail-trematode interactions and parasite community dynamics in aquatic systems: A review. *American Midland Naturalist* **131**(2):209-237

Fenwick, A., Webster, J. P., Bosque-Oliva, E., Blair L., Fleming, F. M., Zhang, Y., Garba, A., Stothard, J. R., Gabrielli, A. F., Clements, A. C., Kabatereine, N. B., Toure, S., Dembele, R., Nyandindi, U., Mwansa, J., Koukounari, A., Webster, P., Bosque-Oliva, E., Blair, L., Fleming, M., Zhang Y., and Koukounari, A. (2009) The

Schistosomiasis Control Initiative (SCI), rationale, development and implementation from 2002-2008. *Parasitology Journal* **13**(6): 17-19.

Frandsen, F., and Christensen, N. O. (1984) An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. *Acta Tropica* **41**(2):181-202.

Frandsen, F. 1979. Studies of the relationship between *Schistosoma* and their intermediate hosts. III. The genus *Biomphalaria* and *Schistosoma mansoni* from Egypt, Kenya, Sudan, Uganda, West Indies (St. Lucia) and Zaire (two different strains, Katanga and Kinshasa). *Journal of Helminthology* **53** (4): 321-48.

French, D., Churcher, S., Gambhir, M., Fenwick, A., Webster, P., Kabatereine, B., and Basanez, G. (2010) Observed reductions in *Schistosoma mansoni* transmission from large-scale administration of praziquantel in Uganda, A mathematical modelling study. *PLoS Neglected Tropical Disease* **4**(11):897-910.

Gelman, A. (2008) Scaling regression inputs by dividing by two standard deviations. *Statistics in Medicine* **27**(15):2865-2873.

Gibson, A. K., Xu, J. Y., and Lively, C. M. (2016) Within-population covariation between sexual reproduction and susceptibility to local parasites. *Evolution* **70**(9):2049-2060.

Gouvras, A. N., Allan, F., Kinung'hi, S., Rabone, M., Emery, A., Angelo, T., Pennance, T., Webster, B., Nagai, H., and Rollinson, D. (2017) Longitudinal survey on the distribution of *Biomphalaria sudanica* and *B. choanomophala* in Mwanza region, on the shores of Lake Victoria, Tanzania: implications for schistosomiasis transmission and control. *Parasites and Vectors* **10**(1):316.

Gray, D. J., McManus, D. P., Li, Y. S., Williams, G. M., Bergquis, R., and Ross, A. G. (2010) Schistosomiasis elimination, lessons from the past guide the future, *Lancet InfectiousDiseases* **10**(1):733.

Greenwood, B., and Mutabingwa, T. (2002) Malaria in 2002. *Nature* **415**(6872):670-672

Grimes, J. E., Croll, D., Harrison, W. E., Utzinger, J., Freeman, M. C., and Templeton, M. R. (2015) The roles of water, sanitation and hygiene in reducing schistosomiasis: a review. *Parasites & Vectors* **8**:156.

Hanelt, B., Mwangi, I. N., Kinuthia, J. M., Maina, G. M., Agola, L. E., Mutuku, M. W., and Mkoji, G. M. (2010) Schistosomes of small mammals from the Lake Victoria Basin, Kenya, new species, familiar species, and implications for schistosomiasis control. *Parasitology Journal* **137**(7):1109 -1118.

Hechinger, R. F., Wood, A. C., and Kuris, A. M. P. (2011) Social organization in a flatworm: trematode parasites form soldier and reproductive castes. Proceedings. Biological Sciences. **7**;278(1706):656-65**7**.

http://www.thiswormyworld.org/maps/distribution-of-s-mansoni-survey-data-in-kenya

Hotez, J., and Fenwick, A. (2009) Schistosomiasis in Africa, An emerging tragedy in our new global Health decade. *PLoS Neglected Tropical Diseases* **3**(9):485.

Hotez, P. J., and 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.

Hotez, J., Engels, D., Fenwick., and Savioli, L. (2010) Africa is desperate for praziquantel, *Lancet,* **376**:496.

Howard, R. S., and Lively C. M. (1994) Parasitism, mutation accumulation and the maintenance of sex. *Nature* **367**(6463):554-557.

Hamburger, J., Hoffman, O., Kariuki, H. C., Muchiri, E. M., Ouma, J. H., Koech, D. K., Sturrock, R. F., and King, C. H. (2004) Large-scale, polymerase chain reaction-based surveillanceof *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya, Anew tool for studying the dynamics of snail infection, *American Journal of Tropical Medicine and Hygiene* **71**(1):765 – 772.

Ibikounlé, M. G., Mouahid, R., Nguéma, M., Sakiti, N. G., Kindé-Gasard, D., Massougbodji, A., and Moné, H. (2012) Life-history traits indicate local adaptation of the schistosome parasite, *Schistosoma mansoni*, to its snail host, *Biomphalaria pfeifferi*. *Experimental Parasitology* **132**(4):501-507.

Jarne, P., and Theron, A. (2001) Genetic structure in natural populations of flukes and snails: a practical approach and review. *Parasitology* **123**:27-40.

Jones-Nelson, O., Thiele, A., and Minchella, J. (2011) Transmission dynamics of two strains of *Schistosoma mansoni* utilizing novel intermediate and definitive hosts. *Parasitology Research* **109**(1):675-687.

Johnson, P. T. J., and Thieltges, D. W. (2010) Diversity, decoys and the dilution effect, how ecological communities affect disease risk. *Journal of Experimental Biology* **213**:961.

Jukes, C., Nokes, A., Alcock, J., Lambo, K., Kihamia, C., Ngorosho, N., Mbise, A., Lorri, W., Yona, E., Mwanri, L., Baddeley, A. D., Hall, A., and Bundy, A. (2002) Partnership for Child Development, Heavy schistosomiasis associated with poor short-term memory and slower reaction times in Tanzanian school children. *Tropical Medicine and International Health* **7** (1):104–117.

Kabatereine, N. B., Brooker, S., Koukounari, A., Kazibwe, F., Tukahebwa, E. M., Fleming, F. M., Zhang, Y., Webster, J. P., Stothard, J. R., and Fenwick, A. (200) Impact of a national helminth control programme on infection and morbidity in Ugandan schoolchildren. *Bulletin of the World Health Organization* **85**(2):91-99.

Kabatereine, N. B., Kazibwe, F., and Kemijumbi, J. 1996. Epidemiology of schistosomiasis in Kampala, Uganda. *East African Medical Journal* **73**(12):795-800.

Kaltz, O., and Shykoff, J. A. (1998) Local adaptation in host-parasite sytems. *Heredity* **81**:361-370.

Katz, N., Chaves, A., and Pellegrino, J. A. (1972) Simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Revista do Instituto de Medicina Tropical São Paulo* **14**:397-400.

Karanja, D. M. S., Awino, E. K., Wiegand, R. E., Okoth, E., Abudho, B. O., Mwinzi, P. N. M., Montgomery, S. P., Secor, W. E. (2017) Cluster randomized trial comparing school-based mass drug administration schedules in areas of western Kenya with moderate initial prevalence of Schistosoma mansoni infections. *PLoS Neglected Tropical Diseases* **11**(10):e0006033.

Karanja, D.M., Colley, D.G., Nahlen, B.L., Ouma, J. H., and Secor, W. E. (1997) Studies on schistosomiasis in western Kenya: I Evidence for immune-facilitated excretion of schistosome eggs from patients with *Schistosoma mansoni* and human immunodeficiency virus coinfections. *American Journal of Tropical Medicine and Hygiene* **56**(5):515-521.

King, C. H., and Dangerfield-Cha M. (2008) The unacknowledged impact of chronic schistosomiasis. *Chronic Illness* **4**(1):65-79.

King, K. C., Delph, L. F., Jokela, J., and Lively, C. M. (2009) The geographic mosaic of sex and the Red Queen. *Current Biology* **15**(17):1438-1441.

King, H., Dickman, K., and Tisch, J. (2005) Reassessment of the cost of chronic helmintic infection, a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* **365** (1):1561-1569.

King, K. C., Jokela, J., and Lively, C. M. (2011) Parasites, sex, and clonal diversity in natural snail populations. *Evolution* **65** (5):1474-1481.

King, C. H. (2015) Health metrics for helminth infections. *Acta Tropica* **141**:150-160.

Kittur, N., Binder, S., Campbell, C. H., King, C. H., Kinung'hi, S., Olsen, A., Magnussen, P., and Colley, D. G. (2017) Defining persistent hotspots: Areas that fail to decrease meaningfully in prevalence after multiple years of mass drug administration with praziquantel for control of schistosomiasis. *American Journal of Tropical Medicine and Hygiene* **97**(6):1810-1817.

Kleiber, C., and Zeileis, A. (2008) *Applied Econometrics with R.* Retrieved from https://CRAN.R-project.org/package=AER

Kloos, H., Fulford, A. J., Butterworth, A. E., Sturrock, R. F., Ouma, J. H., Kariuki, H. C., Thiongo, F. W., Dalton, P. R., and Klumpp, R. K. (1997) Spatial patterns of human water contact and *Schistosoma mansoni* transmission and infection in four rural areas in Machakos District, Kenya. *Social Sciences Medicine* **44**(1):949.

Koskella, B., Vergara, D., and Lively, C. M. (2011) Experimental evolution of sexual host populations in response to parasites. *Evolutionary Ecology Research* **13** (3):315-322.

Kristensen T. K. 1987. A field guide to African freshwater snails. East African species. Second Edition: Danish Bilharziasis Laboratory.

Lafferty, K. D., and Kuris, A. M. (2009) Parasitic castration: the evolution and ecology of body snatchers. *Trends in Parasitology* **25** (12):564-572.

Laidemitt, M. R., Zawadzki, E. T., Brant, S. V., Mutuku, M. W., Mkoji G. M., and Loker, E. S. (2017) Loads of trematodes: discovering hidden diversity of paramphistomoids in Kenyan ruminants. *Parasitology* **144**(2):131-147.

Lange, C. N., Kristensen, T. K., and Madsen, H. (2013) Gastropod diversity, distribution and abundance in habitats with and without anthropogenic disturbances in Lake Victoria, Kenya. *African Journal of Aquatic Sciences* **38** (3):295-304.

Lelo, A. E., Mburu, D. N., Magoma, G. N., Mungai, B. N., Kihara, J. H., Mwangi, I. N., Maina, G. M., Kinuthia, J. M., Mutuku, M. W., and Loker, E. S. (2014) No apparent reduction in schistosome burden or genetic diversity following four years of school-based mass drug administration in Mwea, central Kenya, a heavy transmission area. *PLoS Neglected Tropical Diseases* **8**(10):e3221.

Lively, C. M., and Dybdahl M. F. (2000) Parasite adaptation to locally common host genotypes. *Nature*, **405** (1): 679–681.

Loker E.S. (2013) This de-wormed world?. *Journal of Parasitology* 99:(6):933-942.

Loker, E. S. (2010) Gastropod Immunobiology, Invertebrate Immunity, Edited by Soderhall, New York, K: L. 17-43.

Loker, E. S., Hofkin, B. V., Mkoji, G. M., Mungai, B. N., Kihara, J., and Koech, D. K. (1993) Distributions of freshwater snails in southern Kenya with implications for the biological control of schistosomiasis and other snail-mediated parasites. *Journal of Medicine and Applied Malacology* **5**:1-20.

Loker, E. S., Moyo, H. G., and Gardner, S. L. (1981) Trematode–gastropod associations in nine non-lacustrine habitats in the Mwanza region of Tanzania. *Parasitology* **83**(2):381-399.

Lu, L., Zhang, S. M., Mutuku, M. W., Mkoji, G. M., and Loker, E. S. (2016) Relative compatibility of *Schistosoma mansoni* with *Biomphalaria sudanica* and *B. pfeifferi* from Kenya as assessed by PCR amplification of the *S. mansoni* ND5 gene in conjunction with traditional methods. *Parasites & Vectors* **9**:166.

Magendantz, M. 1972. The biology of *Biomphalaria choanomphala* and *B. sudanica* in relation to their role in the transmission of *Schistosoma mansoni* in Lake Victoria at Mwanza, Tanzania. *Bulletin of the World Health Organization* **47** (3):331-341.

Meredith, J. M., Basu, S., Nimmo, D. D., Larget-Thiery, I., Warr, E. L., Underhill, A., McArthur C. C., Carter, V., Hurd, H., Bourgouin, C., and Eggleston, P. (2011) Site-specific integration and expression of an anti-malarial gene in transgenic *Anopheles gambiae* significantly reduces *Plasmodium* infections. *PLoS Neglected Tropical Diseases* **6**(1):4587.

Morand, S., Manning, S. D., and Woolhouse M. E. (1996) Parasite-host coevolution and geographic patterns of parasite infectivity and host susceptibility. *Proceedings Biological Science* **263** (1366): 119-128.

Mohammed, N. A., Madsen, H., and Ahmed, A. A. (2016) Types of trematodes infecting freshwater snails found in irrigation canals in the East Nile locality, Khartoum, Sudan. *Infectious Diseases of Poverty* **5**:16.

Mutuku, M. W., Dweni, C. K., Mwangi, M., Kinuthia, J. M., Mwangi, I. N., Maina ,G. M., Agola, L. E., Zhang, S. M., Maranga, R., Loker, E. S., and Mkoji, G. M. (2014) Field-derived *Schistosoma mansoni* and *Biomphalaria pfeifferi* in Kenya: a compatible association

characterized by lack of strong local adaptation, and presence of some snails able to persistently produce cercariae for over a year. *Parasites & Vectors* **7**:533.

Mutuku, M., Laidemitt, M. R., Beechler, B. R., Mwangi, I. N., Otiato, F. O., Agola, E. L., Ochanda, H., Kamel, B, Mkoji, G. M., Steinauer, M. L., and Loker, E. S. (2019) A Search for Snail-Related Answers to Explain Differences in Response of *Schistosoma mansoni*to Praziquantel Treatment among Responding and Persistent Hotspot Villages along the Kenyan Shore of Lake Victoria. *American Journal of Tropical Medicine and Hygiene* **101**(1):65-77.

Mutuku, M. W., Lu L., Otiato, F. O., Mwangi, I. N., Kinuthia, J. M., Maina, G. M., Laidemitt, M. R., Lelo, E. A., Ochanda, H., Loker, E. S., and Mkoji, G. M. (2017) A comparison of Kenyan *Biomphalaria pfeifferi* and *B. sudanica* as vectors for *Schistosoma mansoni*, including a discussion of the need to better understand the effects of snail breeding systems on transmission. *The Journal of Parasitology* **103**(6):669-676.

Njenga, S. M., Mutungi, F. M., Wamae, C. N., Mwanje, M. T., Kevin, K., Njiru, K. K., and Bockarie, M. J. (2014) Once a year school-based deworming with praziquantel and albendazole combination may not be adequate for control of urogenital schistosomiasis and hookworm infection in Matuga District, Kwale County, Kenya. *Parasites & Vectors* **7**:74.

Norton, J., Gower, M., Lamberton, L., Webster B. L., Lwambo N. J., Blair L., Fenwick A., and Webster J. P. (2010) Genetic consequences of mass human chemotherapy for *Schistosoma mansoni*, Population structure pre- and post-praziquantel treatment in Tanzania. *American Journal of Tropical Medicine and Hygiene* **83**(1):951.

Odiere, M. R., Rawago, F. O., Ombok, M., Secor, W. E., Karanja, D. M., Mwinzi, P. N., Lammie, P. J., and Won, K. (2012) High prevalence of schistosomiasis in Mbita and its adjacent islands of Lake Victoria, western Kenya. *Parasites & Vectors* **5**:278.

Ofulla, A. V. O., Karanja, D., Omondi, R., Okurut, T., Matano, A., Jembe, T., Abila, R., Boera, P., and Gichuki, J., (2010) Relative abundance of mosquitoes and snails associated with water hyacinth and hippo grass in the Nyanza gulf of Lake Victoria. *Lakes & Reservoirs: Science, Policy and Management for Sustainable Use* **15**(3):255-271.

Okeke, O. C., and Ubachukwu, P. O. (2017) Trematode infections of the freshwater snail *Biomphalaria pfeifferi* from a south-east Nigerian community with emphasis on cercariae of *Schistosoma*. *Journal of Helmintho*logy **9** (3):295-301.

Olsen, A., Kinung'hi, S., and Magnussen, P. (2018) Comparison of the impact of different mass drug administration strategies on infection with *Schistosoma mansoni* in Mwanza region, Tanzania-a cluster-randomized controlled trial. *American Journal of Tropical Medicine and Hygiene* **99**(6):1573-1579.

Onkanga, I. O., Mwinzi, P. N., Muchiri, G., Andiego, K., Omedo, M., Karanja, D. M., Wiegand, R. E., Secor, W. E., Montgomery, S. P. (2016) Impact of two rounds of praziquantel mass drug administration on Schistosoma mansoni infection prevalence and intensity: a comparison between community wide treatment and school based treatment in western Kenya. *International Journal of Parasitology* **46**(7):439-445.

Opande, G. O., Onyango, J. C., and Wagai, S. O. (2004) Lake Victoria: The water hyacinth (*Eichhornia crassipes* [Mart.] Solms), its socio-economic effects, control measures and resurgence in the Winam Gulf. *Limnologica* **34**:105-109.

Plummer, M. L. (2005) Impact of invasive water hyacinth (*Eichhornia crassipes*) on snail hosts of schistosomiasis in Lake Victoria, East Africa. *EcoHealth* **2**(1):81-86.

Prugnolle, F., De Meeus, T., Pointier, J.P., Durand, P., Rognon, A., and The´ron, A. (2006) Geographical variations in infectivity and susceptibility in the host-parasite system *Schistosoma mansoni/Biomphalaria glabrata*, no evidence for local adaptation. *Parasitology* **133**(3):313-319.

Rollinson, D., Knopp, S., Levitz, S., Stothard, J. R., Tchuem Tchuente, L. A., Garba, A., Mohammed, K. A., Schur, N., Person, B., Colley, D. G., and Utzinger, J. (2013) Time to set the agenda for schistosomiasis elimination. *Acta Tropica* **128**(2):423-440.

Ross, A. G., Chau, T. N., Inobaya, M. T., Olveda, R. M., Li Y., and Harn, D. A. (2017) A new global strategy for the elimination of schistosomiasis. *International Journal of Infectious Diseases* **54**:130-137.

Rowel, C., Fred, B., Betson, M., Sousa-Figueiredo, J. C., Kabatereine, N. B., and Stothard, J. R. (2015). Environmental epidemiology of intestinal schistosomiasis in Uganda: population dynamics of *Biomphalaria* (Gastropoda: Planorbidae) in Lake Albert and Lake Victoria with observations on natural infections with digenetic trematodes. *Biomedical Research International* **2015**:717261.

Samuels, A. M., Matey, E., Mwinzi, P. N. M., Wiegand, R. E., Muchiri, G., Ireri, E., Hyde, M., Montgomery, S. P., Karanja, D. M. S., and Secor, W. E. (2012) *Schistosoma mansoni* morbidity among school-aged children: a SCORE project in Kenya. *American Journal of Tropical Medicine and Hygiene* **87**(5):874-882

San Martín, J. L., Brathwaite, O., Zambrano, B., Solórzano, J. O., Bouckenooghe, A., Dayan, G. H., Guzmán, M. G. (2010) The epidemiology of dengue in the Americas over the last

three decades: A worrisome reality. *American Journal of Tropical Medicine and Hygiene* **82**(1):128-135.

Sato, M. O., Rafalimanantsoa, A., Ramarokoto, C., Rahetilahy, A. M., Ravoniarimbinina, P., Kawai, S., Minamoto, T., Sato, M., Kirinoki, M., Rasolofo, V., De Calan, M., and Chigusa, Y. (2018) Usefulness of environmental DNA for detecting *Schistosoma mansoni* occurrence sites in Madagascar. *International Journal of Infectious Diseases* **76**:130-136.

Scolari, F., Siciliano, P., Gabrieli, P., Gomulski, L. M., Bonomi, A., Gasperi, G., and Malacrida, A. R. (2011) Safe and fit genetically modified insects for pest control, from lab to field applications. *Genetica* **139**: 41.

Secor, E. W. (2014) Water-based interventions for schistosomiasis control. *Pathogens and Global Health*. **108**(5):246-254.

Singh, N. D., Criscoe, D. R., and Skolfield, S. (2015) Fruit flies diversify their offspring in response to parasite infection. *Science* **349** (6249):747-750.

Sheldon, B. C., and Verhulst, S. (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* **11**(8):317-321.

Smith, D. H., Pepin J., and Stich A. H. (1998) Human African trypanosomiasis: An emerging public health crisis. *British Medical Bulletin* **54**(2):341-355.

Sokolow, H. S., Wood, C. L., Jones, I. J., Swartz, S. J., Lopez, M., Hsieh, M. H., Lafferty, K. D., Kuris, A. M., Rickards, C., and De Leo, G. A. (2016) Global assessment of schistosomiasis control over the past century shows targeting the snail intermediate host works best. *PLOS Neglected Tropical Diseases* **10**(7):e0004794.

Southgate, V. R., Tchuenté, L. A., Théron, A., Jourdane, J., Ly A., Moncrieff, C., and Gryseels, B. (2000) Compatibility of *Schistosoma mansoni* Cameroon and *Biomphalaria pfeifferi* Senegal. *Parasitology* **121**:501-505.

Southgate, V. R., Brown D. S., Warlow, A., Knowles, R. J., Jones A. (1989). "The influence of *Calicophoron microbothrium* on the susceptibility of *Bulinus tropicus* to *Schistosoma bovis*." *Parasitology Research* **75**: 381.

Spatz, L., Cappa, S. M., and de Nunez, M. O. (2012) Susceptibility of wild populations of *Biomphalaria spp.* from neotropical South America to *Schistosoma mansoni* and interference of *Zygocotyle lunata*. *Journal of Parasitology* **98**(6):1291-1295.

Standley, C. J., Goodacre, S. L., Wade, C. M., and Stothard, J. R. (2014) The population genetic structure of *Biomphalaria choanomphala* in Lake Victoria, East Africa: Implications for schistosomiasis transmission. *Parasites & Vectors* **7**: 524.

Standley, C. J., Vounatsou, P., Gosoniu, L., Jørgensen, A., Adriko, M., Lwambo, N. J.S., Lange, C. N., Kabatereine, N. B., and Stothard, J. R. (2012) The distribution of *Biomphalaria* (Gastropoda: Planorbidae) in Lake Victoria with ecological and spatial predictions using Bayesian modelling. *Hydrobiologia* **683**(1):249-264.

Standley, C. J., Wade, C. M., and Stothard, J. R. (2011) A fresh insight into the transmission of schistosomiasis: A misleading tale of *Biomphalaria* in Lake Victoria. *PLoS One* **6**(10): e26563.

Standley, C. J., Kabatereine, N. B., Lange, C. N., Lwambo, N. J., and Stothard, J. R. (2010) Molecular epidemiology and phylogeography of *Schistosoma mansoni* around Lake Victoria. *Parasitology* **137**(13):1937-49.

Steinauer, M. L., Mwangi, I. N., Maina, G. M., Kinuthia, J. M., Mutuku, M. W., Agola, E. L., Mungai, B., Mkoji, G. M., and Loker, E. S. (2008) Interactions between natural populations of human and rodent schistosomes in the Lake Victoria region of Kenya: a molecular epidemiological approach. *PLoS Neglected Tropical Diseases* **2**(4):e222.

Steinauer, M. L., Hanelt, B., Agola, L. E., Mkoji, G. M., and Loker, E. S. (2009). Genetic structure of *Schstosoma mansoni* in western Kenya: the effects of geography and host sharing. *International Journal of Parasitoly* **39**(12):1353-1362.

Sturrock, F., Klumpp, K., Ouma, H., Butterworth, E., Fulford, C., Kariuki, C., Thiongo, F. W., and Koech, D. (1994) Observations on the effects of different chemotherapy strategies on the transmission of *Schistosoma mansoni* in Machakos District, Kenya, measured by long-term snail sampling and cercariometry. *Parasitology* **109**(1):443.

Tamarith, K. Y., Moore, D. V., DeGiusti, D. L, Ripsom, C. A., and Meleney, H. E. (1994) A Technique for the Perfusion of Laboratory Animals for the Recovery of Schistosomes. *Journal of Parasitology* **33**(5):419-426.

Tavalire, H. F., Blouin, M. S., Steinauer, M. L. (2016) Genotypic variation in host response to infection affects parasite reproductive rate. *International Journal of Parasitology* **46** (2): 123-131.

Theron, A., Coustau, C., Rognon, A., Gourbiere, S., and Blouin, S. (2008) Effects of laboratory culture on compatibility between snails and schistosomes. *Parasitology* **135**(1):1179.

Thompson, J. N. (1994) The Coevolutionary Process. Chicago, University of Chicago Press.

Vergara, D., Jokela, J., Lively, and C. M. (2014) Infection dynamics in coexisting sexual and asexual host populations: Support for the Red Queen hypothesis. *American Naturalist* **184**:22-30.

Webster, J. P., Molyneux, D. H., Hotez, P. J., and Fenwick, A. (2014) The contribution of mass drug administration to global health: past, present and future. *Philosophical Transaction of Royal Society of London Series B Biological Sciences* **369**(1645): 20130434.

Wiegand, R. E., Mwinzi, P. N. M., Montgomery, S. P., Chan, Y. L., Andiego, K., Omedo, M., Muchiri, G., Ogutu, M. O., Rawago, F., Odiere, M. R., Karanja, D. M. S., and Secor, W. E. (2017) A persistent hotspot of *Schistosoma mansoni* infection in a five-year randomized trial of praziquantel preventative chemotherapy strategies. *Journal of Infect Diseases* **216** (11):1425-1433.

World Health Organisation. (2006) Retrieved from World Health Organization: Schistosomiasis and Soil Transmitted Helminths Country Profile. World Health Assembly Resolution **54**: 2006.

World Health Organization.(2013) Retrieved from**.**

http,//www.who.int/mediacentre/factsheets/fs115/en/

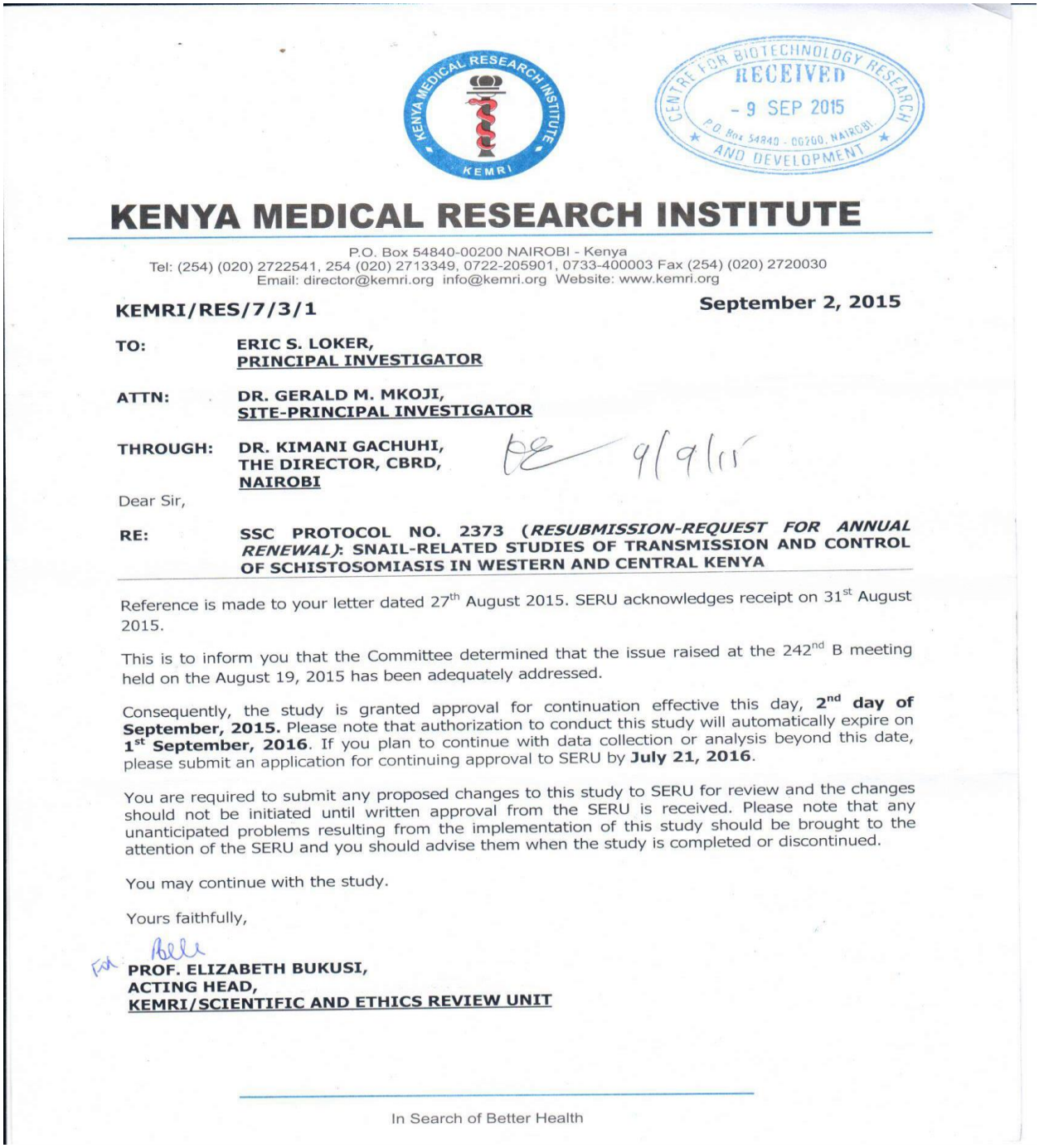
World Health Organization (2014) Retrieved from**.**

http,//www.who.int/mediacentre/factsheets/fs115/en/

Zhang, S. M., Bu, L., Laidemitt, M. R., Lu, L., Mutuku, M. W., Mkoji, G. M., and Loker, E. S. (2018) Complete mitochondrial and rDNA complex sequences of important vector species of *Biomphalaria*, obligatory hosts of the human-infecting blood fluke, *Schistosoma mansoni*. *Scientific Reports* **8**:7341

APPENDICES

Appendix I. KEMRI Scientific and Ethics Review Unit study approval



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 (020) 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2720030
Email: director@kemri.org info@kemri.org Website: www.kemri.org

CENTRE FOR BIOTECHNOLOGY RESEARCH AND DEVELOPMENT
RECEIVED
- 9 SEP 2015
P.O. Box 54840 - 00200, NAIROBI

KEMRI/RES/7/3/1 **September 2, 2015**

TO: **ERIC S. LOKER,**
PRINCIPAL INVESTIGATOR

ATTN: **DR. GERALD M. MKOJI,**
SITE-PRINCIPAL INVESTIGATOR

THROUGH: **DR. KIMANI GACHUHI,**
THE DIRECTOR, CBRD,
NAIROBI

9/9/15

Dear Sir,

RE: **SSC PROTOCOL NO. 2373 (*RESUBMISSION-REQUEST FOR ANNUAL RENEWAL*): SNAIL-RELATED STUDIES OF TRANSMISSION AND CONTROL OF SCHISTOSOMIASIS IN WESTERN AND CENTRAL KENYA**

Reference is made to your letter dated 27th August 2015. SERU acknowledges receipt on 31st August 2015.

This is to inform you that the Committee determined that the issue raised at the 242nd B meeting held on the August 19, 2015 has been adequately addressed.

Consequently, the study is granted approval for continuation effective this day, **2nd day of September, 2015.** Please note that authorization to conduct this study will automatically expire on **1st September, 2016**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to SERU by **July 21, 2016**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from the SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the SERU and you should advise them when the study is completed or discontinued.

You may continue with the study.

Yours faithfully,

Ag Bell

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix II: Kenya Wildlife Service permit to conduct research on snails

KENYA WILDLIFE SERVICE

ISO 9001:2008 Certified

KWS/BRM/5001

17 February 2016

Dr. Gerald Mkoji
Center of Biotechnology Research & Development
Kenya Medical Research Institute
P.O.Box 54840-00200
NAIROBI
e-mail: gmkoji@kemri.org

Dear Dr. Mkoji,

PERMISSION TO CONDUCT RESEARCH ON SNAILS

We acknowledge receipt of your letter dated 17 April 2014 requesting for permission to conduct research on a project titled: '**Snail Related Studies of Transmission and Control of *Schistosomiasis* in Kenya'.** The study will generate data and information that will assist in the prevention and control of *Schistosomiasis* disease transmission to humans in Kenya.

Your team of researchers has been granted permission to conduct the study from **April 2014 – April 2017** upon payment to KWS academic research fees of **Ksh.11,600.** However, you will abide by the set KWS regulations and guidelines regarding the carrying out of research in and outside protected areas. You will also be required to work closely with our Senior Scientist in-charge of Mountain Conservation Area (MCA) and Western Conservation Area (WCA), whom you will give a copy of the progress report on the study.

You will submit a copy of your findings to the KWS Deputy Director, Biodiversity Research and Monitoring on completion of the study.

Yours sincerely,

SAMUEL M. KASIKI, PhD, OGW
DEPUTY DIRECTOR
BIOSDIVERSITY RESEARCH AND MONITORING

Copy to:
- Senior Scientist, MCA
- Senior Scientist, WCA

P.O Box 40241-00100, Nairobi, Kenya. Tel: +254-20-2609233, +254-20-2609234
Wireless: +254-020-2379407-15. Mobile: +254-735 663 421, +254-726 610 508/9. Fax: +254-020-2661923
Email: kws@kws.go.ke Website:www.kws.go.ke

Appendix III: KEMRI Scientific and Ethics Review Unit study approval

KENYA MEDICAL RESEARCH INSTITUTE
CENTRE FOR GLOBAL HEALTH RESEARCH
13 NOV 2015
P.O. Box 1578, KISUMU

KENYA MEDICAL RESEARCH INSTITUTE
CENTRE FOR GLOBAL HEALTH RESEARCH
12 OCT 2015
P.O. Box 1578, KISUMU

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 (020) 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2720030
Email: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **November 04, 2015**

TO: **DR. PAULINE MWINZI,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. STEPHEN MUNGA,**
THE DIRECTOR, CGHR,
KISUMU

FORWARDED
DIRECTOR
GLOBAL HEALTH RESEARCH

Dear Madam,

RE: SSC PROTOCOL No. 1820 (*RESUBMITTED REQUEST FOR ANNUAL RENEWAL*): COMPARISON OF SCHOOL AND COMMUNITY BASED MASS DRUG ADMINISTRATION STRATEGIES FOR CONTROL OF *SCHISTOSOMA MANSONI* IN WESTERN KENYAN AREAS WITH >25% PREVALENCE

Reference is made to your letter dated 22nd October, 2015 of which the KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt on the 27th of October, 2015.

This is to inform you that the Committee determined that the issue raised at the 242nd B meeting of SERU held on 19th August, 2015 has been adequately addressed. Consequently, the study is granted approval for continuation effective this day, **4th day of November, 2015.** Please note that authorization to conduct this study will automatically expire on **3rd November, 2016**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to SERU by **September 23, 2016**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from the SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the SERU and you should advise them when the study is completed or discontinued.

You may continue with the study.

Yours faithfully,

EAB

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix IV: KEMRI Animal Care and Use Committee approval for use of Mice in the study

KENYA MEDICAL RESEARCH INSTITUTE

Centre for Virus Research, P.O.Box 54628 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 254 02 2713349, 0722-205901, 0733-400003 Fax (254) (020) 2726115
Email: cvr@kemri.org

KEMRI/ACUC/ 03.10.15 **19th October 2015,**

Dr. Pauline N. M. Mwinzi
Principal Research Officer,
CGHR-KEMRI

Dr. Mwinzi,

Re: Animal use approval for SSC 1820(Amended): "Comparison of School and Community-based Mass Drug Administration Delivery Strategies for Control of *Schistosoma mansoni* Infections in Western Kenyan in Areas with > 25% Prevalence" protocol

The KEMRI ACUC committee acknowledges the resubmission of a revised version of above mentioned protocol. It has been confirmed that all the issues raised earlier have been addressed appropriately and acknowledges that the use of laboratory mice is justified in achieving the study objectives.

The committee grants you the approval to use laboratory animals in your study but recommends that you proceed with the study only after obtaining the final approval from the KEMRI scientific and ethics review unit (SERU).

Approval is granted for a period of two years starting from when the SERU approval will be obtained. If you still intend to use laboratory animals after the initial approval, you are required to submit an application for continuing approval to the ACUC 1 month prior to the expiry of the initial SERU approval.

The committee expects you to adhere to all the animal handling procedures as described in the protocol. The committee wishes you all the best in your work.

Yours sincerely,

Dr. Konongoi Limbaso
Chairperson KEMRI ACUC

KENYA MEDICAL RESEARCH INSTITUTE
★ 19 OCT 2015 ★
ANIMAL CARE AND USE COMMITTEE
Signature: .....

Appendix V: NEMA permit to conduct research on snails

(r. 12.)

Third Schedule (r. 12)

nema
mazingira yetu | uhai wetu | wajibu wetu

Application Reference No: **NEMA/AGR/46/2014**
Registration: **0066**

Access Permit

This permit is hereby granted to **Dr. Gerald M. Mkoji, Kenya Medical Research Institute (KEMRI) P, O Box 54840-00200 NAIROBI** in accordance with regulation 11 of the Environmental Management and Co-ordination (Conservation of Biological Diversity and Resources, Access to Genetic Resources and Benefit Sharing) Regulations, 2006 for the collection of the following genetic resources:
Biomphalaria Snails Species, the Intermediate Host of the Bilharzia Parasite, Schistosoma Mansoni in Fresh Waters of Kisumu and Kirinyaga Counties

located at:

Lake Victoria in Kisumu Mwea Tebere Irrigation Scheme in Kirinyaga.

This permit is issued subject to the Regulations and all agreements concluded pursuant to its grant, and may be suspended, cancelled or revoked should the holder breach any of those agreements and the conditions of issue and those contained in the Regulations.

Dr. Gerald M. Mkoji being the holder of this permit, including his agents and assignees, undertake to abide by the conditions of this permit and to promptly report to the National Environment Management Authority any matter that may prejudice the interests of Kenya and other parties concluded pursuant to the grant of this permit.

Signed: ..... Date: 15-05-2017
Director General,
National Environment Management Authority

P.T.O.

ISO 9001 : 2008 Certified

Appendix VI: NACOSTI permit to conduct research

Permit No. : NACOSTI/P/16/9609/12754

Date Of Issue : 2nd August,2016

Fee Recieved :USD 1035

THIS IS TO CERTIFY THAT:

DR. ERIC SAMUEL LOKER

of UNIVERSITY OF NEW MEXICO, 609

Lajolla Place-87123 Albuquerque,

NM,has been permitted to conduct

research in Homabay , Kirinyaga ,

Kisumu Counties

on the topic: SNAIL-RELATED STUDIES

OF TRANSMISSION AND CONTROL OF

SCHISTOSOMIASIS IN WESTERN AND

CENTRAL KENYA

for the period ending:

30th July,2017

Applicant's

Signature

Director General

National Commission for Scien

Technology & Innovation

Appendix VII: Informed consent documents for study participants

Project Title: Snail-Related Studies of Transmission and Control of Schistosomiasis in western and central Kenya

Investigators: Eric S. Loker, Department of Biology, University of New Mexico, Albuquerque, USA; Gerald M. Mkoji, Centre for Biotechnology Research and Development, Kenya Medical Research Institute, Nairobi, Kenya

Introduction: Dr. Gerald Mkoji of the Kenya Medical Research Institute (KEMRI) and Dr. Eric Loker of the University of New Mexico (UNM), USA are carrying out a study on bilharzia, a disease caused by worms that are transmitted through water snails, and which enter the human body by going through the skin. The purpose of the study is to enable us understand better the role snails play in transmitting the bilharzia parasites, and to see how we can develop ways to destroy the bilharzia parasites that occur in the snail host, as means to control bilharzia. Bilharzia makes millions of people living in areas of warm climate, sick, and children suffer most from the disease. Even though, medication is available to get rid of the infection in the body, people still continue to pick the bilharzia parasites when they visit water bodies that have snails that carry the bilharzia parasites. We have chosen to carry out this study in this area because we know that bilharzia snails and bilharzia parasites occur in the water bodies of this area. To carry out this study, we need to enroll school children into the study. However, we will need permission from parents/guardians to include their children in the study. From each child included in the study, with permission of the parent/guardian, we will ask for a small amount of stool so that we can check if they have bilharzia. The children found to have bilharzia will be asked to give an additional stool from which we can isolate eggs of bilharzia parasites for our experiments. All the children found to have bilharzia or

other intestinal parasites will be given medication to get rid of the infections, free of charge. However, any child found or suspected to have other medical conditions will be referred to the nearest health centre or hospital for medical care, but their parents/guardians will have to meet the medical expenses for these other medical conditions. As a parent/guardian, we requested to permission for your child to take part in this study. Taking part in the study is voluntary and no can force or influence you to get your child to take part in the study. Even when you give permission, your child can still leave the study, if you or your child decides to do so, at any time, in the future, without suffering any penalty or losing the benefits available for him or her through this study. Please take time to read this information sheet about the study, and when you have read, feel free to ask questions or to seek clarification, now or later, on any issue you do not understand about this study or about your child's participation in the study. We also want you to know that this study has been approved by the KEMRI and the UNM.

Purpose of the Study: The purpose of this study is to enable us understand better the role snails play in transmitting the bilharzia parasites, and to see how we can develop ways to destroy the bilharzia parasites that occur in the snail host, as means to control bilharzia.

Procedures to be Used: School children, aged 6-15 years whose parents/guardians give written permission for their children to take part in the study will be included in the study. All participating children, regardless of age, will also be asked to give assent (to accept), if they want to take part in the study. If you give permission, your child will be enrolled in the study, a doctor will examine your child, he or she will then be asked to give a small stool sample to enable us check if he or she has bilharzia. If he or she is found to have bilharzia we may ask him/her to give some more stool so that we can isolate eggs of bilharzia parasite for use in our experiments. We also want you to know that the eggs or bilharzia parasites obtained from the stool your child provides, may be sent

to the University of New Mexico (UNM) where additional analysis and research will be done on the parasite. If your child is found bilharzia or any other instestinal parasites from the stool examination, he or she receive medication for bilharzia (praziquantel) and/or medication for the other intestinal parasites.

Benefits: Your child is found to have bilharzia or other intestinal parasitic infections he/she will receive medication for these ailments, under the supervision of a qualified doctor, free of charge. If other medical conditions are discovered or suspected in your child, he/she will be referred a health centre or a hospital near you, for further medical attention. However, you will be expected to meet the medical expenses for these other ailments, for your child.

Risks, Hazards and Discomforts Associated with the Procedures: Giving stool samples should not cause any harm in your child. The medications your child will receive to treat bilharzia or other parasitic infections your child may have, are known to be safe and should not cause any harm. However, in some people, these medications may cause some side effects, which may include dizziness, headaches, and stomach pain, but these, are mild and last only for a brief period.

Confidentiality: The identity and test results of your child will be kept confidential, and he or she will be given an identification number, and your child or results of the tests done will be referred to or identified using the number he or she will be given, even in any correspondence, reports or publications related to this study. All the information and records about your child will remain confidential, and will be kept in a lockable cabinet at KEMRI, and only authorized personnel carrying out this study will have access to these.

Contacts for Further Information: If you need more information about this study, please contact: Dr. Gerald Mkoji, CBRD, KEMRI, PO Box 54840-00200; Office Phone: 020-2717131 or Cell

Phone: 0721-585 696, or by e-mail: gmkoji@kemri.org If you have questions about the rights of your child as a research participant, please contact: The Secretary, KEMRI Ethics Review Committee, PO Box 54840-00200, Nairobi; Phone: 020-2722541, 0722-205901, 0733-400003; e-mail: erc@kemri.org

Storage, Exportation of Samples and Further Studies: From the stool samples you give, we will remove eggs of bilharzia parasites and hatch them into larvae, which we need in our experiments. Some of these larval parasites or the resulting adult worms obtain in this study may be sent to Dr. Eric Loker's Laboratory at the University of New Mexico (UNM) in America, where further analysis of the parasites will be carried out.

Informed Consent Agreement for Parents/Guardians

I, Mr./Mrs/Miss _____, being an adult aged 18 years and over, and being the parent/guardian of:Master/Miss (Child's Name) _____ _____ Aged_____,who attends_____ _____School, do hereby give permission to Prof/Dr./Mr./Mrs/Miss _____ _____for my child to take part in the new study known as "Snail-Related Studies of Transmission and Control of Schistosomiasis in Western and Central Kenya" which has been explained to me in _____, a language I speak fluently and understand clearly, and now, I know what the study is all about, the tests to be done on my child, the benefits my child will receive for taking part in the study, the medications he/she will be given, if found to be sick with bilharzia or other intestinal illnesses caused by parasites, the side effects he/she could suffer from the medication, which I have been told, are mild, temporary, and should not cause any harm to my child. I was given an opportunity to ask questions and to seek clarifications of the

issues I had not understood clearly about the study, and I am satisfied with the answers and the explanations I was given. I have also, been told that if I have additional questions or concerns about the study later, I can contact the researcher in charge of the study, and if I have questions or concerns about my child's rights as a participant in this study, I can contact: The Secretary, KEMRI's Ethics Review Committee, Kenya Medical Research Institute (KEMRI), P.O. Box 54840-00200, Nairobi, Phone: 020-2722541, 0722-205901, 0733-400003; e-mail: erc@kemri.org

I accept my child to take part in this study, and agree that he/she can give stool samples for the tests needed in this study. I have been told that my child can leave the study any time he/she decides to do so, and I have been assured that he/she will not suffer any penalty or loss of benefits that he/she should get through this study. All these things have been explained to me and my child in _____, a language we speak fluently, and understand clearly. I agree to allow the researchers to remove the eggs of the bilharzia parasites from the stool samples my child will give, and they can take these eggs or the resulting adult worms, abroad for further investigations and research.

_____

Signature (or Thumb Print) of Parent/Guardian

_____

Date

_____

Name of the Person Obtaining Consent and Signature

_____

Name and Signature (or Thumb Print) of Witness

Informed Consent Agreement for Parents/Guardians in Dholuo

Yie mar jonyuol/jorit

An, Mr./Mrs./Miss: _____,kaka ngato ma jahigni 18 kata mangeny, kendo kaka janyuol/jarit_____ma hike gin_____ ma dhi e _____ skul, ahero yie chiwo thuolo kuom somo ma iluongo ni “Somo ma omakore gi ogonglo mar tuo mar aremo ka luore gi kaka tuo e geng’o kod kaka tuo medore ei piny Kenya” ma ose lerna dholuo,dhok ma awacho kendo awinjo malar. Gise lerona wach somoni, pim ma gi biro timo kuom nyathina, yuto ma nyathi nyalo yudo e somo kod yiedhe ma nyathi biro yudo ka en kod tuo mar bilharzia kata touche mamoko mag ich ma ikelo kod kute, kata chanruook moko ma nyalo yudo nyathi, ma awinjo ni gin ma yomyom kendo ma ok dag aminga, ose miya thuolo mar golo penjo kata dwaro ler kuom gik ma ok awinjo maber kendo amor kod duoko kaachiel kod weche ma ler ma omiya. Bende onyisa ni bang’e ka an kod penjo kata yore mamoko kaluwre kod adiera maga to anyalo chopo kuom: Jagoro, KEMRI’s Echics Review Committee, Kenya Medical Reseach Institue (KEMRI), P.O. Box 54840-00200, Nairobi, Simu: 020-2722541, 0722-205901, 0733-400003; e-mail: erc@kemri.org

Ayie mondo nyathina obed jakanyo mar somoni kendo ayie onyalo chiwo oko mare kuom pim madwarore e somoni, onyisa ni onyalo wuok e somo saa asya ka ohero kendo onge kum ma obiro yudo kata wito yuto ma onyalo yudo ka okalo e somoni. Gigi duto ose lerna kaachiel kod nyathina gi dholuo, dhok ma awacho kendo awinjo maler. Ayie weyo jogo ma timo nonro mondo ogol tonge kute bilhazia e oko ma nyathina ochiwo kendo ginyalo tero gi e pinje mamoko kuom pim kod nonro ma matut.

_____

Seyi (kata lwedo ma thuon) mar Janyuol/Jarit

_____

Tarik

_____

Nying Ngato Mandiko Yieruok

_____

Nying kod Seyi mar Janeno

Consent Agreement for Adults (18 years and over) in Dholuo

Yieruok koum jomandongo (higni 18 ka dhi nyine)

An, Mr. /Mrs./Miss_____kaka ngato moromo higni 18 ka dhi nyime kendo anyalo ngado paro kenda ka adonjo kata ok adonjo e somoni, amiyo Prof./Dr./Mr./Mrs./Miss:_____ thuolo mar keta e somo ma gi timo miluongo ni "Somo ma omakore gi ogonglo mar tuo mar aremo ka luore gi kaka tuo e geng'o kod kaka tuo medore ei piny Kenya" ma gi se lerona kod dholuo, dhok ma awacho kendo awinjo maler, omiyo sani angeyo gima en. Gise lerona wach somoni, pim ma gi biro timo kuoma, yuto ma anyalo yudo e somo kod yiedhe ma abiro yudo ka an kod tuo mar bilharzia kata touche mamoko mag ich ma ikelo kod kute, kata chanruook moko ma nyalo yuda, ma awinjo ni gin ma yomyom kendo ma ok dag aminga, ose miya thuolo mar golo penjo kata dwaro ler kuom gik ma ok awinjo maber kendo amor kod duoko kaachiel kod weche ma ler ma omiya. Bende onyisa ni bang'e ka an kod

penjo kata yore mamoko kaluwre kod adiera maga to anyalo chopo kuom: Jagoro, KEMRI's Ethics Review Committee, Kenya Medical Reseach Institue (KEMRI), P.O. Box 54840-00200, Nairobi, Simu: 020-2722541, 0722-205901, 0733-400003; e-mail: erc@kemri.org

Ayie bedo jakanyo mar somoni kendo ayie chiwo oko mara kuom pim madwarore e somoni, onyisa ni anyalo wuok e somo saa asya ka ahero kendo onge kum ma abiro yudo kata wito yuto ma anyalo yudo ka okalo e somoni. Gigi duto ose lerna kod dholuo, dhok ma awacho kendo awinjo maler. Ayie weyo jogo ma timo nonro mondo ogol tonge kute bilhazia e oko ma achiwo kendo ginyalo tero gi e pinje mamoko kuom pim kod nonro ma matut.

_____

Seyi (kata lwedo ma thuon mar jakanyo)

_____

Seyi mar janeno

NYING NGATO MA OCHUNG NI SOMO:_____

NYING MAR KAR TICH:_____

Appendix VIII: First publication abstract in Journal of Parasitology

J. Parasitol., 103(6), 2017, pp. 669–676

A COMPARISON OF KENYAN *BIOMPHALARIA PFEIFFERI* AND *B. SUDANICA* AS VECTORS FOR *SCHISTOSOMA MANSONI*, INCLUDING A DISCUSSION OF THE NEED TO BETTER UNDERSTAND THE EFFECTS OF SNAIL BREEDING SYSTEMS ON TRANSMISSION

Martin W. Mutuku, Lijun Lu*, Fredrick O. Otlato†, Ibrahim N. Mwangi, Joseph M. Kinuthia, Geoffrey M. Maina, Martina R. Laidemitt*, Eric A. Lelo, Horace Ochanda‡, Eric S. Loker*, and Gerald M. Mkoji

Centre for Biotechnology Research and Development, Kenya Medical Research Institute, P.O. Box 54840-00200, Nairobi, Kenya. Correspondence should be sent to Martin W. Mutuku at: *mmutuku@kemri.org*

ABSTRACT: In Kenya, schistosomes infect an estimated 6 million people with >30 million people at risk of infection. We compared compatibility with, and ability to support and perpetuate, *Schistosoma mansoni* of *Biomphalaria pfeifferi* and *Biomphalaria sudanica*, 2 prominent freshwater snail species involved in schistosomiasis transmission in Kenya. Field-derived *B. pfeifferi* (from a stream in Mwea, central Kenya) and *B. sudanica* (from Nawa, Lake Victoria, in western Kenya) were exposed to *S. mansoni* miracidia isolated from fecal samples of naturally infected humans from Mwea or Nawa. Juvenile (<6 mm shell diameter), young adult (6–9 mm), and adult snails (>9 mm) were each exposed to a single miracidium. *Schistosoma mansoni* developed faster and consistently had higher infection rates (39.6–80.7%) in *B. pfeifferi* than in *B. sudanica* (2.4–21.5%), regardless of the source of *S. mansoni* or the size of the snails used. *Schistosoma mansoni* from Nawa produced higher infection rates in both *B. pfeifferi* and *B. sudanica* than did *S. mansoni* from Mwea. Mean daily cercariae production was greater for *B. pfeifferi* exposed to sympatric than allopatric *S. mansoni* (583–1,686 vs. 392–1,232), and mean daily cercariae production among *B. sudanica* were consistently low (50–590) with no significant differences between sympatric or allopatric combinations. Both non-miracidia–exposed and miracidia-exposed *B. pfeifferi* had higher mortality rates than for *B. sudanica*, but mean survival time of shedding snails (9.3–13.7 wk) did not differ significantly between the 2 species. A small proportion (1.5%) of the cercariae shedding *B. pfeifferi* survived up to 40 wk post-exposure. *Biomphalaria pfeifferi* was more likely to become infected and to shed more cercariae than *B. sudanica*, suggesting that the risk per individual snail of perpetuating transmission in Kenyan streams or lacustrine habitats may differ considerably. High infection rates exhibited by the preferential self-fertilizing *B. pfeifferi* relative to the out-crossing *B. sudanica* point to the need to investigate further the role of host breeding systems in influencing transmission of schistosomiasis by snail hosts.

Vector-borne diseases including malaria, dengue, Zika virus, and trypanosomiasis continue to pose major challenges to public health (Smith et al., 1998; Greenwood and Mutabingwa, 2002; San Martín et al., 2010). Similarly, snail-transmitted infections also remain problematic in the developing world, and although snails are not vectors in a more conventional sense in that they do not bite their hosts to perpetuate transmission, they play an indispensable role in transmission and are considered to be vectors by the WHO (2016).

With a few exceptions, digeneans (digenetic trematodes or flukes) use snails as first intermediate hosts, enjoying a remarkably productive period of asexual reproduction within snails that culminates with the production of cercariae that may continue for months and in some cases over a year (Mutuku et al., 2014). The prolonged production and release of numerous cercariae into the environment gives the life cycles of human-infecting schistosomes considerable stability, thereby challenging control efforts. Given the vast populations of snails that occupy many natural transmission sites, control of snail-transmitted diseases is a formidable challenge. When schistosomiasis control has been most successful is when snail control has been implemented (Lelo et al., 2014; Njenga et al., 2014; Sokolow et al., 2016), highlighting the importance of knowing more about the biology of the snail hosts and their interactions with snail-transmitted parasites of human and veterinary concern.

The competence of snails to serve as hosts for schistosomes is influenced by several different factors including, but not limited to, infection prevalence as measured by the proportion of schistosome-exposed snails that actually produce and release (shed) cercariae, the length of time required to complete sporocyst development for the first release of cercariae following exposure to infection (the pre-patent period), the longevity of infected snails, duration of actual shedding of the schistosome-exposed snails, and daily output of cercariae from infected snails (Ibikounlé et al., 2012). It is also important to appreciate that schistosome snail hosts exist in complex environmental settings that can influence their capacity to support transmission. They must simultaneously cope with exposure to potential infection with several other species of digenetic trematodes, which may even be more common than schistosomes (Loker et al., 1981; Mohammed et al., 2016) and that also have the potential to cause castration, thereby strongly affecting fitness of the snails. Moreover, infection with other trematode species may alter susceptibility to infection with schistosomes (Spatz et al., 2012). Finally, the suitability of snail environments often varies dramatically with season (Charbonnel et al., 2005), which is

Received 5 May 2017; revised 27 June 2017; accepted 7 July 2017.

* Department of Biology, Parasitology Division, Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico 87131.

† Centers for Disease Control and Prevention, Nairobi P.O. Box 606-00621, Nairobi, Kenya.

‡ School of Biological Sciences, College of Biological and Physical Sciences, University of Nairobi, Kenya, P.O. Box 30197, Nairobi, Kenya.

DOI: 10.1645/17-72

Appendix IX: Second publication abstract in American Journal of Tropical Medicine and Hygiene

Am. J. Trop. Med. Hyg., 101(1), 2019, pp. 65–77
doi:10.4269/ajtmh.19-0089

A Search for Snail-Related Answers to Explain Differences in Response of *Schistosoma mansoni* to Praziquantel Treatment among Responding and Persistent Hotspot Villages along the Kenyan Shore of Lake Victoria

Martin W. Mutuku,[1,2]* Martina R. Laidemitt,[3] Brianna R. Beechler,[4] Ibrahim N. Mwangi,[1] Fredrick O. Otiato,[5] Eric L. Agola,[1] Horace Ochanda,[2] Bishoy Kamel,[3] Gerald M. Mkoji,[1] Michelle L. Steinauer,[6] and Eric S. Loker[3]

[1]*Centre for Biotechnology Research and Development, Kenya Medical Research Institute, Nairobi, Kenya;* [2]*School of Biological Sciences, College of Biological and Physical Sciences, University of Nairobi, Nairobi, Kenya;* [3]*Department of Biology, Center for Evolutionary and Theoretical Immunology, Museum of Southwestern Biology, Parasitology Division, University of New Mexico, Albuquerque, New Mexico;* [4]*Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon;* [5]*Influenza Surveillance Program, Centers for Disease Control and Prevention, Nairobi, Kenya;* [6]*Department of Basic Medical Sciences, Western University of Health Sciences, Lebanon, Oregon*

Abstract. Following a 4-year annual praziquantel (PZQ) treatment campaign, the resulting prevalence of *Schistosoma mansoni* was seen to differ among individual villages along the Kenyan shore of Lake Victoria. We have investigated possible inherent differences in snail-related aspects of transmission among such 10 villages, including six persistent hotspot (PHS) villages (≤ 30% reduction in prevalence following repeated treatments) located along the west-facing shore of the lake and four PZQ-responding (RESP) villages (> 30% prevalence reduction following repeated treatment) along the Winam Gulf. When taking into account all sampling sites, times, and water hyacinth presence/absence, shoreline-associated *Biomphalaria sudanica* from PHS and RESP villages did not differ in relative abundance or prevalence of *S. mansoni* infection. Water hyacinth intrusions were associated with increased *B. sudanica* abundance. The deeper water snail *Biomphalaria choanomphala* was significantly more abundant in the PHS villages, and prevalence of *S. mansoni* among villages both before and after control was positively correlated with *B. choanomphala* abundance. Worm recoveries from sentinel mice did not differ between PHS and RESP villages, and abundance of non-schistosome trematode species was not associated with *S. mansoni* abundance. *Biomphalaria choanomphala* provides an alternative, deepwater mode of transmission that may favor greater persistence of *S. mansoni* in PHS villages. As we found evidence for ongoing *S. mansoni* transmission in all 10 villages, we conclude that conditions conducive for transmission and reinfection occur ubiquitously. This argues for an integrated, basin-wide plan for schistosomiasis control to counteract rapid reinfections facilitated by large snail populations and movements of infected people around the lake.

INTRODUCTION

One of the most prevalent and persistent of the world's neglected tropical diseases is human schistosomiasis.[1] There is currently considerable momentum to bring schistosomiasis under control and to proceed to elimination efforts.[2–4] An essential weapon in the elimination of schistosomiasis is the drug praziquantel (PZQ), which has been used extensively in a variety of control programs and has considerably reduced prevalence and intensity of infection.[5] There is also a growing appreciation for the need for integrated control programs taking into account sanitation, provision of safe water, education, and acknowledging the fundamental role played by freshwater snails as vectors of the disease.[6,7] The persistent success of schistosome parasites is owed substantially to their molluscan hosts, which support the prolific production of human-infective cercariae and that often exist in huge populations across a variety of freshwater habitats.

Africa harbors 90+% of the global burden of *Schistosoma mansoni*.[8] In Kenya, there are three taxa of *Biomphalaria* snails that perpetuate transmission: 1) *Biomphalaria pfeifferi*, whose distribution includes tributaries feeding Lake Victoria, and in small impoundments and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along the coast; 2) *Bicmphalaria sudanica*, mainly found along the shores of Lake Victoria and Lake Jipe and their surrounding swamps; and 3) *Biomphalaria choanomphala*, a deeper water inhabitant of Lake Victoria.[9,10] The latter two taxa are likely members of the same species and are frequently referred to as ecophenotypes or ecomorphs.[11–13]

Prevalence of intestinal schistosomiasis in Kenya is highest (> 50%) in the Mwea Irrigation Scheme in central Kenya and in the Lake Victoria basin in western Kenya.[14] Recent studies assessing the prevalence of *S. mansoni* infection in school children in the lake basin reported overall prevalences of 60.5% and 69%, with villages on lake islands having 2-fold higher prevalence rates than mainland villages.[15,16] Schools closer to the lake also had higher prevalence rates than those further from the lake.[15] Both higher prevalences and likelihood of reinfection following treatment would logically be associated with more frequent contact with lake water containing infected snails.[17]

Several chemotherapy-based initiatives have been undertaken to control schistosomiasis in the Lake Victoria region.[17–20] Among them, the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) project implemented school-based and village-wide mass drug administration (MDA) in varying treatment regimens over four consecutive years (2011–2015) for 150 Kenyan villages situated on or near the eastern shore of Lake Victoria.[17] Villages were randomized with respect to six different treatment strategies (study arms), which involved combinations of school-based or community-wide treatments, with three study arms receiving annual treatment and three receiving treatment twice over a 4-year period. Regardless of study

*Address correspondence to Martin W. Mutuku, Centre for Biotechnology Research and Development, Kenya Medical Research Institute, P.O. Box 54840-00200, Nairobi, Kenya. E-mail: mmutuku@kemri.org