EVALUATION OF A NOVEL PRIMARY PREVENTION TECHNIQUE FOR THE CONTROL OF UROGENITAL SCHISTOSOMIASIS: A PILOT INTERVENTION IN ADASAWASE, GHANA

A Dissertation

submitted by

Karen Claire Kosinski

In partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in

Civil and Environmental Engineering

TUFTS UNIVERSITY

August 2011

ADVISOR: DAVID M GUTE, PhD, MPH

ABSTRACT

In Adasawase, a rural Ghanaian town, 36% of girls (n=43/119) and 51% of boys (n=69/134) who were screened three times were infected with *S. haematobium* as of 2008. Praziquantel was administered by Ghana Health Services. In 2009, schoolchildren were screened three or more times and prevalence was estimated to be 13% (n=13/98) among girls and 24% (n=29/122) among boys, indicating ongoing transmission of *S. haematobium*. In 2009, children again received praziquantel. Children in Adasawase contract schistosomiasis in the Tini River where they play, bathe, and collect water. An intervention in the form of a novel water recreation area (WRA) was designed and implemented to function as an effective, sustainable form of primary prevention. It consists of a concrete pool supplied by two hand-pumped boreholes and a rainwater collection system. Adasawase was selected for the study based on the relatively high prevalence of urogenital schistosomiasis, strong support of the project by community leaders, and water availability. Concrete structural elements were constructed in 2008 and waterproofing and aesthetic touches were completed in 2009. The WRA was opened for public use after praziquantel treatment in 2009. Community members operate and maintain the WRA. Local children were encouraged by community leaders to use the WRA instead of the Tini River for recreation.

In 2010, one year after opening the WRA, children were screened at least three times for *S. haematobium* eggs; only 2% of girls (n=2/105) and 5% of boys (n=7/141) were positive, reflecting annual incidence after WRA construction. Risk factors associated with infection also changed significantly during the course of the study. Age, sex, and observed river use in 2009 correlated with 2008 infection status. Observed river use and previous infection status were the only significant risk factors in 2009. Statistical analysis of positive children in 2010 was not

possible due to the small number of positive children. The WRA should be evaluated in other water-rich regions to determine whether it is effective and sustainable in other settings. Continued praziquantel administration is still necessary for morbidity control and should be promoted in conjunction with WRAs.

ACKNOWLEDGEMENTS

I wish to thank David M. Gute, my thesis advisor, for an inexpressibly wonderful experience as I pursued my doctoral degree. Throughout the five years in which we have worked together, he has been unflaggingly kind, supportive, enthusiastic, and patient. I appreciate his great faith in my abilities as a researcher and as a person, especially at the beginning of my fieldwork when I spent a substantial amount of time in Ghana. I continue to be impressed by the amount of effort that David puts into coordinating a heterogeneous doctoral committee stretched across several continents. David is a role model who consistently maintains perspective on the relative importance of research, teaching, family, friends, and life. I will always remain grateful that David willingly supported and encouraged my interest in teaching over the years. David is a great mentor, a great researcher, a great listener, and a great friend.

I am thankful for the unique and invaluable contributions from each of my thesis committee members. Dr. Miguel Stadecker spent countless hours patiently discussing various study designs as we considered potential community partners. Miguel has been remarkably enthusiastic and supportive for the duration of my time as a doctoral candidate. Dr. Jeanine Plummer provided me with tremendous guidance and support that extended well beyond the call of duty. I am grateful for her meticulous approach to research, her faith in me, her willingness to train me long before I was officially her student, and for her friendship. Dr. John Durant has consistently asked the interesting and provocative questions that have helped substantially strengthen the research overall. He also performed the difficult task of first visiting Ghana and establishing our research connections in the Eastern Region. Dr. Kwabena Bosompem provided expert parasitological advice and helped provide us with a strong sense of Ghanaian culture.

Boss is also the strongest promoter of community-directed development whom I have ever met; for this, he is to be commended.

I thank Michael Nii Adjei, known to many as 'Tom'. Tom ranks among the truest friends I have ever had, and I have had many. To Tom, I owe my ability to conduct fascinating and gratifying research in a beautiful country full of hospitable people. I owe him boundless thanks for translating both languages and Ghanaian culture for me, for his outstanding public relations efforts with the schoolchildren and with other community members, and for his superb commitment to the success of the project. I cannot imagine having carried out my research without him.

I thank Anjuli Wagner and Jonny Crocker, two dear friends who both gave many months of their lives to the project. In Ghana and in the United States, I especially cherish their generosity, their senses of humor, and their willingness to work collaboratively for something in which they believed. I also thank Dickson Osabutey who spent a substantial portion of time in the field with our team as the laboratory technician. He is one of the funniest people I have ever met; Osa is one of those rare individuals who promotes team bonding through the sheer force of his fantastic, effervescent personality. I remain grateful to him for the opportunity to learn quite a lot about practicing public health research in a field setting.

I thank the Chief of Adasawase, Osabarima Kwame Tia II, as well as the Assemblyman, Hon. Alex Asamoa-Bonti, and the entire council of elders in Adasawase, Ghana. I am touched by their faith and trust in me and I hope that I have been able to confer a benefit to their community. I also thank the children of Adasawase for humoring me when I interrupted their school day repeatedly for three years to ask them to pee in cups. I thank them for finding our work interesting and somewhat entertaining! I thank their teachers for assisting our study team with

maintaining organization and order and for putting up with disruptions to their classrooms on a fairly regular basis.

The following individuals deserve recognition for their contributions to fieldwork and/or construction in Ghana: Mr. Felix Agyei, Mr. Elijah Bosompem, Mr. Kofi Danquah, Mr. Kwame Anim, Mr. Ernest Asante, Mr. Yaw Asante, Mr. Samuel Tetteh, Mr. Kwasi Obeng, Mr. Daniel Adom, Mr. Quincy Moore, Ms. Carrie Ellsworth, Ms. Victoria Mason, Ms. Karalee Conover-Fowler, and Ms. Mavis Akyaamaa.

I thank Dr. Jill Rulfs, whom I have known since my freshman year of college at Worcester Polytechnic Institute. Jill has remained a role model and friend over the years and provided substantial support in the final year of my fieldwork in Ghana. Dr. Bill Baller has also provided encouragement and support for the past thirteen years as I have pursued three different degrees and have traveled to a number of far-off locales. Bill Baller is one of the people who champions me most strongly and with the most unfailing enthusiasm.

For moral support, logistic support, and friendship, I have a substantial number of individuals to thank. Although I do not list everyone here, their good deeds will always be remembered gratefully. First, I thank my family: especially my mom. I also thank all of my friends at Tufts, but in particular, Rhiannon and Mary, who have been with me since the beginning. I thank Wayne Chudyk, Rachel Szyman, Jeff Griffiths, Mark Woodin, Barbara Parmenter, and all of the other staff and faculty members at Tufts who have made my life as a graduate student both intellectually challenging and enjoyable.

TABLE OF CONTENTS

CHAPTER 1: BACKGROUND – UROGENITAL SCHISTOSOMIASIS

Global Distribution of Schistosomiasis

Schistosomiasis is 1 of 13 Neglected Tropical Diseases (NTDs); the disease affects an estimated 207 million people worldwide (Steinmann et al. 2006), but there is little accurate data on infection prevalence (Engels et al. 2002, King 2010, van der Werf et al. 2003b). King (2010) suggests that between 391 and 587 million people are infected. The World Health Organization (WHO) estimates that at least 160 million people in sub-Saharan Africa alone suffer from the disease and 779 million are at risk of contracting it (WHO 2002). King et al. (2006) estimate that schistosomiasis is responsible for 6 to 13.5 million disability adjusted life years (DALYs).

Schistosomiasis is endemic in 76 countries worldwide (Engels et al. 2002), 46 of which are in Africa (Steinmann et al. 2006). Of the 46 endemic countries in Africa, 29 countries have over 1 million infected people (Steinmann et al. 2006). Improved estimates of the worldwide distribution of schistosomiasis are needed (Engels et al. 2002, King 2010, van der Werf et al. 2003b). Currently, the worldwide prevalence of morbidity is estimated via extrapolation of locally-collected infection prevalence data (Ratard et al. 1992, van der Werf et al. 2003b). However, the prevalence of infection with *S. haematobium* is often determined by screening individuals once via either a blood dipstick test for hematuria or via a filtration test for *S. haematobium* eggs (van der Werf et al. 2003b). These methods may result in an underestimation of infection prevalence due to temporal and spatial variation of blood and eggs in urine. Moreover, schistosomiasis is a focal disease that is often underappreciated at the national level "since aggregated reports from larger regions may not identify foci of high prevalence" and the

true distribution of infection may be distorted (WHO 1993). The number of cases of schistosomiasis reported through national health care systems may also cause underestimation of infection prevalence because not all infected individuals present at clinics; infected individuals may be even less likely to report if they believe that treatment is unavailable (WHO 1993). It is likely that worldwide schistosomiasis prevalence estimates are too low (King 2010).

Schistosomiasis is the generic name for diseases caused by blood flukes in the genus *Schistosoma*. Schistosomes belong to the class *Trematoda* of the phylum *Platyhelminthes*. *Schistosoma japonicum* and *S. mansoni* are primarily responsible for intestinal schistosomiasis and *S. haematobium* is primarily responsible for urogenital schistosomiasis. Urogenital schistosomiasis caused by *S. haematobium* affects an estimated 112 million people worldwide, which is roughly twice as many people (54 million) as are affected by *S. mansoni* (King 2005, van der Werf et al. 2003b). Over 15% of the population living in West Africa is estimated to have hematuria due to urogenital schistosomiasis at any given time (van der Werf et al. 2003b). Collectively, "Kenya, Ghana, Mozambique, Tanzania and Nigeria are predicted to account for more than 50% of the morbidity cases associated with *S. haematobium*, partly due to their large populations" (van der Werf et al. 2003b). Urogenital schistosomiasis has been reported in Ghana since 1895 (Doumenge et al. 1987) and it is the focus of this pilot intervention study. The other clinical varieties of schistosomiasis, as caused by other species of the parasite, are not considered in this dissertation. Here, 'schistosomiasis' refers to urogenital schistosomiasis unless otherwise stated.

Pathology Associated with Urogenital Schistosomiasis

Pathology associated with *S. haematobium* can result from chronic or acute infection. Although some forms of morbidity are related to the intensity of infection, others are independent of worm burden and many are difficult to assess accurately, especially given long time lags between infection and the onset of severe disease (King 2005). Intensity of infection is defined by the WHO (2003b) as follows: light infection is 1 to 49 eggs/10ml urine, heavy infection is equal to or greater than 50 eggs/10ml urine. In practice, when individuals with schistosomiasis are screened more than once, they may present with widely varying egg counts at each screening (Hatz et al. 1990). In such cases, it is not clear whether an average, a maximum, a minimum, or some other measure of an egg count is to be used to categorize the individual as "lightly" or "heavily" infected.

Data relating intensity of infection and morbidity/mortality attributable to schistosomiasis are lacking (Hatz et al. 1990, van der Werf et al. 2003b). As of 2005, there was no consensus about which long- and short-term morbidities should be attributed to schistosomiasis (King 2005). It is estimated that 50 to 60% of people *without* severe schistosomiasis have symptoms; the rest of the individuals without severe schistosomiasis may be asymptomatic (WHO 1993). The number of symptomatic and asymptomatic individuals is considered by the WHO (1993) "a public health problem of enormous proportions".

Acute schistosomiasis, or Katayama Fever, presents as a systemic hypersensitivity reaction to the schistosome larvae, called schistosomulae (Gryseels et al. 2006). Acute disease typically manifests in travelers from non-endemic areas who are exposed to water containing infective forms of the parasite (Gryseels et al. 2006). Chronic schistosomiasis, sometimes referred to as "Bilharzia", is primarily caused by a pro-inflammatory host reaction to eggs

trapped in soft tissue. Trapped eggs secrete antigens that are irritating to the host, and in an effort to contain eggs and their antigens, granulomas may form that are hundreds of times the size of the egg. Over time, if these granulomas are replaced by fibrotic tissue, blood flow can be obstructed to major organs and organ function can be compromised (Coon 2005, Gause et al. 2003, Gryseels et al. 2006). The WHO (2002) states that in areas where schistosomiasis-related morbidity is to be controlled, health care systems must be equipped to handle cases of acute and chronic schistosomiasis. Acute and chronic schistosomiasis are discussed in further detail below.

Acute Infections

Pathology associated with acute schistosomiasis can manifest abruptly, usually weeks to months after exposure to contaminated water (Gryseels et al. 2006), but can occur as rapidly as several days after exposure (Lambertucci 2005). "The disease starts suddenly with fever, fatigue, myalgia, malaise, non-productive cough, eosinophilia, and patchy infiltrates on chest radiography. Abdominal symptoms can develop later" (Gryseels et al. 2006). In most individuals with acute infections, symptoms disappear in a few months without treatment, but some patients experience more serious and longer-lasting sequelae (Gryseels et al. 2006, Coon 2005). It is not clear whether adult worms survive the immune response, or die when symptoms disappear. Moreover, the reasons that Katayama Fever does not typically occur in populations living in endemic areas are not clear. Gryseels et al. (2006) report that Katayama Fever may be under-diagnosed among people in endemic areas, or that *in utero* sensitization to schistosome antigens may be protective against developing an acute reaction to the parasite later in life.

Chronic Infections

A rigorous definition of chronic urogenital schistosomiasis infection has not been found. Here, chronic infection refers to the state of infection among people who carry *S. haematobium* worms but who are not experiencing Katayama Fever due to acute schistosomiasis. These individuals may or may not be symptomatic and may or may not have eggs in their urine. Studies of the immunopathology of schistosomiasis have shown that the mere presence of schistosomes, in addition to infection intensity, is an important determinant of chronic morbidity (King 2010).

Chronic infection is usually characterized by the morbidity associated with an immune response to schistosome eggs trapped in tissue (Gryseels et al. 2006), which can be insidious in nature. Host genetics, not worm burden, seem to be the most important factor in determining whether an individual will develop severe pathology (Gause et al. 2003). Individuals with urinary tract morbidity due to schistosomiasis may not present with eggs, or eggs may simply be undetected by screening tests that lack sensitivity (Hatz et al. 1990, King 2010).

Morbidity due to Chronic Infection

In the case of chronic infection with *S. haematobium*, bladder walls can thicken and develop polyps in response to egg-induced inflammation. "Often, adult worms and multiple eggs can be found at the base of the polyps" Coon (2005). Polyps may rupture, allowing blood to leak into the lumen of the bladder; macro- and microscopic blood in the urine are clinical symptoms of urogenital schistosomiasis. In addition to thickening of the bladder walls, severe long-term pathology associated with urogenital schistosomiasis includes calcification of the bladder walls, urethral obstruction, kidney inflammation, kidney failure, bladder cancer, and death (Coon 2005, Gryseels et al. 2006, Parkin 2008). In 2003, van der Werf et al. (2003b) estimated that in sub-

Saharan Africa, 88 million (90%CI 67 - 102) people have "minor bladder wall pathology" and that an additional 18 million (90%CI 5.1 – 27) have "major bladder wall pathology" (van der Werf et al. 2003b).

The disability associated with such moderate disease burden is receiving more attention recently than it did in past years. Schistosomiasis can be a "poverty trap", given the limited family budgets of many who live in poor, rural areas (King et al. 2006, King 2010, Molyneux et al. 2005). As of 2003, there was insufficient evidence to characterize some of the more subtle potential morbidities associated with urogenital schistosomiasis, such as growth and cognitive developmental effects and reductions in physical fitness (van der Werf et al. 2003b, King 2010). Thus, these morbidities are not included in available morbidity estimates.

Pathology due to *S. haematobium* has been assessed in a number of studies. In a group of 533 schoolchildren in Tanzania (age range 7 – 17 years), 77% presented with eggs at least once during 5 days of testing, and urinary tract pathology was observed via ultrasound in 67.2% of the population (Hatz et al. 1998). This population of children was characterized as "heavily infected", with 51.1% of children having maximum egg counts (over 5 days of testing) of ≥ 50 eggs/10ml of urine (Hatz et al. 1998). Van der Werf et al. (2003b) estimate that 63% of people infected with *S. haematobium* have hematuria during any given 2-week period, 28% had dysuria (painful urination), and 8.5% had major hydronephrosis (kidney swelling). In endemic areas, urinary tract pathology is rarely caused by diseases other than urogenital schistosomiasis (Hatz et al. 1998). Ultrasound has been used with schistosomiasis mansoni to assess liver and spleen pathology. It is important to note that "The absence of gross or ultrasound detectable fibrosis does not exclude the possible presence of severe morbidity in *S. mansoni* infected patients" (Vennervald and Dunne 2005). Presumably, this is also true for urogenital schistosomiasis:

techniques for detecting pathology are imperfect and not all individuals affected by severe morbidity are detected by screening programs.

Moderate pathology due to chronic schistosomiasis affects a larger number of people than does organ damage and death, and may represent a greater threat to overall public health (Secor 2005, King et al. 2006, King 2010). Moderate pathology includes anaemia, fatigue, stunting, malnutrition, or cognitive development problems (Secor 2005, King 2010, WHO 1993). Anemia may result from leakage of blood into the lumen of the bladder, or through schistosome-induced inflammation (Secor 2005). Children with *S. haematobium* infections who present with greater than 200 eggs/10ml urine may lose as much blood per month as a menstruating woman (Stephenson et al. 1985, as cited in Secor 2005). While heavy and moderate infections with *S. haematobium* clearly cause host morbidity, it has also been recognized that "any level of schistosome infection is associated with significant inflammation in the human host, leading to non-trivial subclinical morbidity in some, and premature mortality among a small proportion of those infected" (King 2005).

A survey conducted in 2000 in sub-Saharan Africa showed that 70 million of 682 million people reported (presumably visible) hematuria and 32 million reported dysuria (WHO 2006). Thickening of the bladder walls and hydronephrosis as a result of urogenital schistosomiasis affected 18 million and 10 million people, respectively (WHO 2006). King et al. (2004) found that among people (n = 3,178) in rural Kenya aged 2 to 100 years, 46% were passing *S. haematobium* eggs, 8.6% were passing more than 400 eggs/10ml of urine, 51% presented with hematuria, 14% had bladder abnormalities as detected by ultrasound, and 1.2% had "moderate-to-severe hydronephrosis by World Health Organization criteria". In this population,

hydronephrosis affected younger (age range 5 to 24 years) and older (≥ 45 years) members of the population disproportionately.

In rural Ghana, Shiff et al. (2006) evaluated an individual's likelihood of developing bladder cancer by comparing *S. haematobium* egg counts, abnormalities of bladder walls, and the presence or absence of a pair of biomarkers. The first biomarker, BLCA-4, is a protein strongly associated with cancer. The other biomarker used is a score assigned by a process called quantitative nuclear grading (QNG). The method involves staining and quantification of nuclear abnormalities in bladder epithelium cells. BLCA-4 and QNG are considered sensitive and specific for bladder cancer or as markers for cancer precursors. With increasing age and parasite burden, Shiff et al. found that bladder abnormalities and bladder cancer biomarkers tended to increase. Startlingly, nearly 40% of individuals chronically infected with *S. haematobium* were positive for BLCA-4. A high mean QNG score, positive BLCA-4 results, and bladder damage diagnosed with ultrasound scans correlated well with each other (Shiff et al. 2006). The worldwide estimate of bladder cancer cases due to *S. haematobium* infection was assessed by Parkin (2008). Worldwide, the number of cases attributable to urogenital schistosomiasis is approximately 10,600, which is roughly 3% of the total worldwide burden of bladder cancer (Parkin 2008). It is likely that this represents an underestimate of cancer cases, given the method used to estimate the number of individuals at risk (extrapolation of data collected in locally-appropriate surveys).

The disability and disease burden of chronic schistosomiasis infection was reassessed by King et al. (2005) and a significant association was found between chronic infection and anemia, under-nutrition, pain, exercise intolerance, and diarrhea. Heavy *S. haematobium* infections (≥ 50eggs/10ml urine), as compared with light infections (< 50 eggs/10ml urine), are significantly

associated with low hemoglobin levels. Low hemoglobin levels (< 120 g/L) correlate with impaired field productivity (3 to 33%), a 60% decrease in peak exercise capacity, and a peak work endurance reduction of 8 to 20%. In contrast to earlier speculation, no significant association was found between infection and school performance, or infection and infertility, but few studies address these possible correlations (King et al. 2005).

Infection with schistosomes is often accompanied by a skewing of the immune system towards an 'anti-inflammatory' response (see *Acquired and Innate Immunity to Schistosome Infection and Schistosomiasis-Related Pathology*). This shift in immune response protects against severe schistosomiasis-related pathology, but can result in poor and inappropriate responses to viral infections (Secor 2005).

Mortality due to Chronic Schistosomiasis

In sub-Saharan Africa alone, more than 200,000 deaths result from schistosomiasis-induced kidney failure or haematemesis (vomiting blood) (Utzinger et al. 2003); revisions of mortality estimates suggest that up to 280,000 deaths can be attributed to schistosomiasis in sub-Saharan Africa (Gryseels et al. 2006). Mortality due to *S. haematobium* typically results from kidney failure and bladder cancer (van der Werf et al. 2003b). In sub-Saharan Africa, an estimated 1.7 million people have a non-functioning kidney due to schistosomiasis (predicted incidence 180,000 and annual mortality 150,000) (Van der Werf et al. 2003b). Estimates of mortality depend on a number of factors, namely: "the accuracy of the associations between prevalence of infection and morbidity, the quality of the available prevalence of infection data and the chosen degree of heterogeneity in prevalence of infection" (van der Werf et al. 2003b).

Age-dependent Pathology

The prevalence of schistosomiasis, hematuria, and *S. haematobium* eggs in urine all tend to increase throughout childhood and peak between ages 10 and 20 as a function of contact with infected water (Bradley and McCullough 1973, Gryseels et al. 2006, Hatz et al. 1990, Lima e Costa 1987, Mafiana et al. 2003, Ndyomugyenyi and Minjas 2001, Tucker 1983, Wilkins et al. 1979). Although parasitological indicators (eggs in urine, hematuria) tend to peak in the second decade of life, Hatz et al. (1998) found that urinary tract pathology (as assessed by ultrasound) reappeared in a cohort of Tanzanian schoolchildren (n = 244) after treatment with praziquantel, and that the reappearance of pathology was not age-dependent. It is not clear whether this trend would be seen had children involved in the study been selected randomly; the 244 children studied were those not lost to follow-up (6 assessments of morbidity over 24 months) from an initial pool of 533. This study design may have inadvertently selected for children with more serious disease than other local children. Hatz et al. (1998) found that morbidity among young children (7 – 9 years) was "substantial" and suggest treating children in this age group regularly.

It is not clear whether infection prevalence and associated morbidity decrease after the second decade of life. Evidence suggests that bladder pathology as a result of long-term schistosomiasis infection may be largely underestimated in adults (Shiff et al. 2006). While worm burden and egg excretion may decrease with increasing age, some adults continue to carry chronic light infections that are difficult to detect parasitologically. The reasons for tapering worm burden with age are not entirely clear but may be due to changes in immunity and/or behavior (Gryseels et al. 2006). These chronic light infections may cause serious complications in people approximately 35 to 50 years old (Shiff et al. 2006).

Differences in Pathology between Males and Females

Within a community, the prevalence of schistosomiasis, the risks associated with contracting the infection, and infection intensity may be dependent on sex; this is likely to be the case when females and males have different water contact patterns. As described above, pathology is not necessarily related to worm or egg burden. The risk of developing severe pathology is based on a number of factors, including host genetics and the presence of co-infections, but it may also be sex-dependent. The results of several case studies are described that demonstrate the heterogeneity among various communities.

In a Nigerian study by Mafiana et al. (2003), males and females showed no difference in disease prevalence by visible hematuria (17.7% of tested children) or egg count (71.8% of tested children). King et al. (2004) found that among Wadigo people in rural Kenya, males tended to have "significantly heavier mean infection levels and greater odds of having morbidity (heavy infection, hydronephrosis, or bladder abnormalities) than did females", based on the results of a logistic regression analysis. Similarly, Ndyomugyenyi and Minjas (2001) found that among children aged 5 to 19 years in a Tanzanian cohort (n = 483), boys had significantly higher infection intensities (54 versus 38 eggs/10ml urine, via one egg count, $p = 0.001$) and a higher overall prevalence of infection (54.6% versus 40.8%, $p = 0.004$) than did girls. Hatz et al. (1998) report that among Tanzanian schoolchildren (n = 533, age range 7 to 17 years), girls over 12 years tended to have a higher prevalence of pathologic urinary tract lesions due to *S. haematobium* infection than did younger girls (statistical significance not assessed). It does not appear as though there was an age-dependent pattern for boys. The most serious type of urinary tract lesions were found more often in boys than in girls ($\chi^2_1 = 4.86$, $p = 0.03$). Egg counts and urine blood tests by reagent dipstick "did not differ significantly between the two sexes", a

surprising result given that pathology did differ (Hatz et al. 1998). These findings could indicate that boys in the two communities respond less appropriately (i.e. produced a pro-inflammatory response) to schistosome infection than do girls or that the relationship between egg output and worm burden differs between the sexes (i.e. more eggs remain trapped in tissue in boy children, causing greater pathology).

Women of child-bearing age are considered one of the groups at high risk of schistosomiasis-related morbidity, given the risk of developing anemia in addition to the loss of blood to menstruation (Olds 2005). Women are at risk of complications due to genital schistosomiasis, an area of study that is receiving increasing attention. Genital schistosomiasis may predispose women to an increased risk of transmission of sexually transmitted infections (STIs). Genital schistosomiasis can be difficult to diagnose, given that many affected women do not present with eggs in their urine (Vennervald and Dunne 2005). *S. haematobium* eggs have been detected in the semen of infected men, but as with women, these infections may be difficult to diagnose in adults (Vennervald and Dunne 2005). Genital schistosomiasis in both sexes appears to be highly underestimated (Bruun and Aagaard-Hansen 2005).

Schistosomiasis and HIV/AIDS Co-infections

Pathology related to the host immune reaction to *S. haematobium* eggs can be severe, but the increased risk of contracting other infections, such as human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS) and Sexually Transmitted Infections (STIs), can be a more serious consequence of infection (Gryseels et al. 2006). Experiments conducted on Rhesus Macaques showed that animals infected with schistosomes prior to challenge with a simian version of HIV are infected with the virus at a dose 17 times lower than schistosome-free

animals. Further, peak viral loads and viral replication increase in co-infected animals. The authors suggest worldwide helminth control as a possible avenue to blunt HIV/AIDS transmission (Chenine et al. 2008).

Harms and Feldmeier (2005) reviewed the relationship between genital schistosomiasis and HIV. Indirect evidence suggests that genital schistosomiasis "may be a risk factor for the transmission of HIV"; genital schistosomiasis is found in over half of the women with urinary schistosomiasis. Genital schistosomiasis causes lesions of the vulva, vagina, and cervix; associated pathology includes "thinning, erosion, and ulceration of the epithelium, particularly in the cervix. These lesions reflect a break in the integrity of the mucosal barrier and may increase the risk [to women] of HIV infection and transmission" (Harms and Feldmeier 2005). The number of CD4+ T cells typically increases at sites of schistosome-induced granulomas, which predisposes women to infection with HIV, as the virus is bound by receptors on these cells. Men are at increased risk of transmitting HIV to their partners if they are co-infected with schistosomes, due to the chronic inflammation caused by *S. haematobium* eggs in the region of the prostate and seminal vesicles (Harms and Feldmeier 2005).

The HIV-1 virus "replicates more readily in activated T cells, especially those with a Th2 phenotype" (Maggi et al. 1994, as cited in Secor 2005), which is the phenotype that is most common and most appropriate for responding to schistosome infection, and to helminth infections in general (Secor 2005). In studies reviewed by Secor (2005), humans co-infected with schistosomes and HIV in Kenya and Uganda did not experience a reduction in HIV load following treatment of schistosome infections, which was surprising (Secor 2005). Other studies suggest that risk of HIV transmission from males to females is probably greater in individuals co-infected with *S. haematobium*. Males who are co-infected have increased numbers of

lymphocytes (white blood cells) in their semen than do males without schistosomiasis; lymphocytes contain the virus. Females carrying *S. haematobium* may have genital lesions that erode the protective physical barrier and increase their risk of contracting HIV (Secor 2005, Vennervald and Dunne 2005). Moreover, the Th2 environment that typically accompanies schistosome infection may precede the increased presence of activated lymphocytes, which are the targets of HIV (Secor 2005).

Pathology in individuals co-infected with HIV and *S. mansoni* may include increased destruction of the liver, as compared with individuals infected with *S. mansoni* alone. This is due to the HIV-positive host's decreased ability to form granulomas (via cell-mediated immune responses) around schistosome eggs embedded in tissue, and the subsequent leakage of cytotoxic egg products (Lambertucci 2005). Because an appropriate host immune response is required by the schistosome egg to travel through tissue and exit the body, few or no eggs may be found in urine or fecal samples in immunocompromised individuals (Lambertucci 2005). In such cases, the efficacy of praziquantel is expected to decrease since the drug requires a competent immune response in order to kill adult worms (Lambertucci 2005). Despite this reality, treatment of schistosomiasis was effective in patients co-infected with HIV and *S. mansoni* or *S. haematobium* (Karanja et al. 1998 and Mwanakasale et al. 2003 as cited by Harms and Feldmeier 2005).

Schistosomiasis and Malaria Co-infections

Increased attention is being paid to malaria-schistosomiasis co-infections, given the overlap in geographic distribution of the two diseases in much of the tropics and especially in sub-Saharan Africa. In 2005, the Scientific Working Group on Schistosomiasis called for additional research

on malaria-schistosomiasis co-infections, especially as they relate to "cognition, work capacity, and anaemia" (WHO 2005). In the same publication, Vennervald and Dunne (2005) describe the lack of knowledge about hepatosplenomegaly and the difficulty of collecting accurate data about hepatosplenomegaly in co-endemic areas. Additional research should be conducted about severe hepatosplenic disease in co-infected individuals (Vennervald and Dunne 2005).

Acquired and Innate Immunity to Infection and Schistosomiasis-Related Pathology

Little is known about the mechanisms by which individuals become resistant to infection with schistosomes (WHO 2005). This is an emerging field of research, and while some studies in the murine (mouse) model and in human populations have begun to uncover clues about the nature of the human immune response, much work remains to be done before the basic aspects of pathology and infection are understood. For example, it is not clear how, to what extent, and in whom schistosomes regulate the human immune system for the purpose of ensuring their own survival. It is also unclear why some individuals experience severe morbidity while others suffer only minor morbidity. Finally, the identification of biological markers that characterize individuals at high risk of severe pathology would be useful from the perspective of screening for long-term, serious adverse outcomes (WHO 2005).

Immune Response to Schistosome Larvae

When a human host is first invaded by schistosome larvae, the host immune response is almost negligible. This is in contrast to many other types of helminth infections and may reflect the long history of human-schistosome interaction. Migrating and developing schistosome larvae are capable of down-regulating the human immune response by triggering the production of anti-

inflammatory molecules, such as interleukin-4 (IL-4) and IL-10. In addition to down-regulating the inflammatory immune response, schistosome larvae coat their tegument with host proteins in an initially successful attempt to appear non-foreign to the human immune system. Eventually, the immune system will detect the larvae, but recognition typically does not result in worm death. However, recognition of schistosome larvae offers the benefit of creating a more robust response to future challenges by other schistosomulae and may help to protect somewhat against reinfection (Gause et al. 2003).

Pro-inflammatory and Anti-inflammatory Immune Responses and Host Protection

Adult worms and eggs elicit immune responses that tend to be more pro-inflammatory than those induced by larvae. Pro-inflammatory molecules are necessary for the development of schistosome worms and for the migration of eggs through tissue. A protective response is associated with the initial development of a pro-inflammatory Th1 reaction that gives way to an anti-inflammatory Th2 reaction after approximately 5 weeks. With the anti-inflammatory response comes the production of non-complement fixing antibodies, primarily immunoglobulin G (IgG) and IgE. If the Th1 response does not shift to a Th2 response, the host may develop high pathology, which is characterized by severe inflammation and organ damage. Interleukin 10 (IL-10) has been shown to be critical in down-regulating the Th1 response; mice that lack IL-10 develop severe disease and large egg-induced granulomas. Up-regulation of the Th2 response is related to the production of antibodies against schistosome antigens; most of these antigens are found on schistosome egg carbohydrates and glycolipids (Gause et al. 2003).

Severe forms of schistosomiasis japonica in the human host have been linked to two genes, each of which codes for a molecule prominent in the human immune system. The first

gene codes for human leukocyte antigen (HLA) class II molecules and the second for the interleukin (IL)-13 promoter. The specific structure of HLA class II molecules dictates the degree to which CD4+ T cells are activated to respond to schistosome antigens. Thus, it has been hypothesized that even small changes in the structure of the HLA class II genes could be responsible for large increases in liver pathology, as seen in some schistosomiasis japonica patients. Hirayama (2005) reports that his research group has found that some of the HLA class II gene haplotypes are significantly associated with increased or decreased liver fibrosis. The underlying mechanism for how HLA class II molecules actually present schistosome antigens to CD4+ T cells remains to be determined (Hirayama 2005).

One particular IL-13 haplotype has been significantly linked to liver fibrosis. Although the IL-13 allele is inherited independently of the HLA class II allele, Hirayama (2005) found that individuals with both HLA class II and IL-13 high-pathology alleles have much higher odds ratios of liver fibrosis than patients with only one allele, which may suggest relatively complex interaction mechanisms (Hirayama 2005).

Age-Related Acquired and Innate Immunity

Gryseels et al. (2006) describe findings from several studies that suggest that age-related acquired immunity plays a role in schistosomiasis reinfection. More specifically, reinfection rates are much greater in children than in adults, but variations in water contact patterns do not sufficiently explain these observations, suggesting a learned immune response to adult or larval-stage worms. Innate immunity may also play a role in preventing reinfection among older members of a population. In studies of populations only recently exposed to schistosomes,

children tend to be more infected than adults, despite the fact that neither group has had the opportunity to develop an acquired immune response (Gryseels et al. 2006).

Resistance to Reinfection with Schistosomes Following Treatment with Praziquantel

Treatment of infection with praziquantel correlates with at least a temporary up-regulation of IL-4, IL-5, and IL-13. These cytokines are associated with a decreased risk of reinfection with schistosomes. However, children are known to produce smaller amounts of these cytokines post-treatment than do adults, which may partly explain the age-dependent pattern of resistance to re-infection (Secor 2005).

Hereditary Component of Immunity to Schistosome Pathology

King et al. (2004) found that among Wadigo people in rural villages in Kenya, familial relationships explain little of the variation in pathology caused by infection with *S. haematobium*. The Wadigo are believed to have been exposed to *S. haematobium* for at least several centuries. The lack of correlating between familial ties and pathology is in contrast to studies conducted in Brazil, Senegal, and Sudan. King et al. (2004) reviewed papers that suggest that loci on chromosomes 5 and 6 are responsible for some of the high pathology associated with *S. mansoni* infection in these countries. However, among the Wadigo in Kenya, presence of infection and infection intensity were based on the results of "duplicate determination of *S. haematobium* egg counts per 10ml of urine…using a standard Nuclepore…filtration technique", but it was not clear whether the egg counts per 10ml of urine were taken from the same parent sample, or whether urine was collected on 2 different days and tested separately (see *Diagnosis*

of Urogenital Schistosomiasis). Urine egg counts are known to exhibit temporal variation and are not considered reliable indicators of worm burden (Hall 1982).

Schistosome-Mediated Immune Response: Effects on Vaccination and Response to other Pathogens

Children infected with schistosomes have been found to respond less robustly to vaccination against some types of pathogens (ex. tuberculosis) than do children without schistosomiasis (King et al. 2006, King 2010). This is likely due to the skewing of the immune response towards a Th2 phenotype during helminth infections, while a strong Th1 response is required for some types of immunity (King et al. 2006).

In many tropical areas, schistosomiasis and malaria are co-endemic (Hartgers and Yazdanbakhsh 2006, Hartgers et al. 2008). For protection against pathology associated with malaria infection, a host must mount a strong Th1 response that is followed by production of malaria-specific antibodies (Hartgers et al. 2008). Because schistosomes typically induce a Th2 response, studies have begun to address the need for more information about host immune responses when challenged by both types of parasite simultaneously. Studies of animal models of malaria-schistosomiasis co-infection have produced results that are difficult to interpret given variations in parasite species, host species, and infection timing (*Plasmodium* infection first, *Schistosoma* infection first, chronic versus acute infections, time lapse between induced infection with either species, etc.). A more complete overview of these studies can be found in Hartgers and Yazdanbakhsh (2006).

In studies of malaria-schistosome co-infections in human hosts, results have also been seemingly contradictory, but the age of study populations, the species of parasites involved, the

geographic study area, and the parasite loads varies, making comparisons difficult. The range of malaria-specific outcomes was wide. In some cases, malaria parasitaemia decreased or was unaffected. In other cases, malaria parasitaemia increased, splenomegaly increased, or both parasitaemia and the presence of fever above 38ºC increased. Studies of *S. haematobium* infection in children in Senegal and Mali have shown some protective effects of light schistosome infection (1 – 9 eggs/10ml urine and < 50 eggs/10ml urine, respectively) on malaria outcomes but these studies remain to be confirmed elsewhere; in the study carried out in Mali, the effects were only present among children aged 4 to 8 years (Hartgers and Yazdanbakhsh 2006). All of these studies point to the need for further research in the area of helminth-malaria co-infections; it cannot be assumed that prevention and treatment of helminth infections in malaria-endemic areas is unequivocally beneficial until additional data is collected.

> "A cautious conclusion that can be drawn from the human studies conducted so far, is that helminth infections seem to increase the susceptibility to malaria infection and light symptoms such as fever, whereas they might protect against some complications of severe malaria. In the acute phase of *Schistosoma* infection, the immune response is of a Th1 type and might boost the Th1 response necessary to fight *Plasmodium spp.*, but increase the inflammatory response, causing severe malaria. However, after egg deposition, the immune response in schistosome-infected individuals becomes Th2-skewed, which might interfere with the development of an adequate Th1 response necessary to control parasitaemia and the resultant malaria attacks" (Hartgers and Yazdanbakhsh 2006).

In a pilot study in Ghana, Hartgers et al. (2008) described IL-10 production in response to malarial antigens. The production of IL-10 *in vitro* via blood samples taken from helminth-infected children was higher than in children not infected with helminths ($p = 0.06$), but the sample size was small ($n = 20$) and data were not stratified by presence/absence of co-infection with malaria (11 of 20 were malaria-positive by microscopy) (Hartgers et al. 2008). More

research about the effect of helminth infections on malaria pathology and parasitaemia is needed, especially in light of the attempts being made to control or eradicate parasitic helminths.

Studies reviewed by Hartgers and Yazdanbakhsh (2006) showed that atopic reactions to house dust mites increased in Venezuelan and Gabonese children following treatment for helminth infections. Other studies showed that infection with schistosomes or *Onchocerca volvulus* caused individuals to mount less appropriate immune responses to vaccination with tetanus or BCG than their uninfected counterparts (Hartgers and Yazdanbakhsh 2006). The T cell responses of individuals co-infected with *S. mansoni* and hepatitis C virus were not as robust as the T cell responses of individuals infected with hepatitis C virus alone (Hartgers and Yazdanbakhsh 2006).

Schistosoma haematobium Life Cycle

S. haematobium infection is normally contracted through skin contact with tropical fresh water bodies that harbor the parasite and its intermediate host, the snail. The life cycle (Figure 1) begins when the eggs of *S. haematobium* are released into a fresh water body via human urine. Eggs are typically 60 x 140 – 170 μm in size and possess a characteristic terminal spine (WHO 1994).

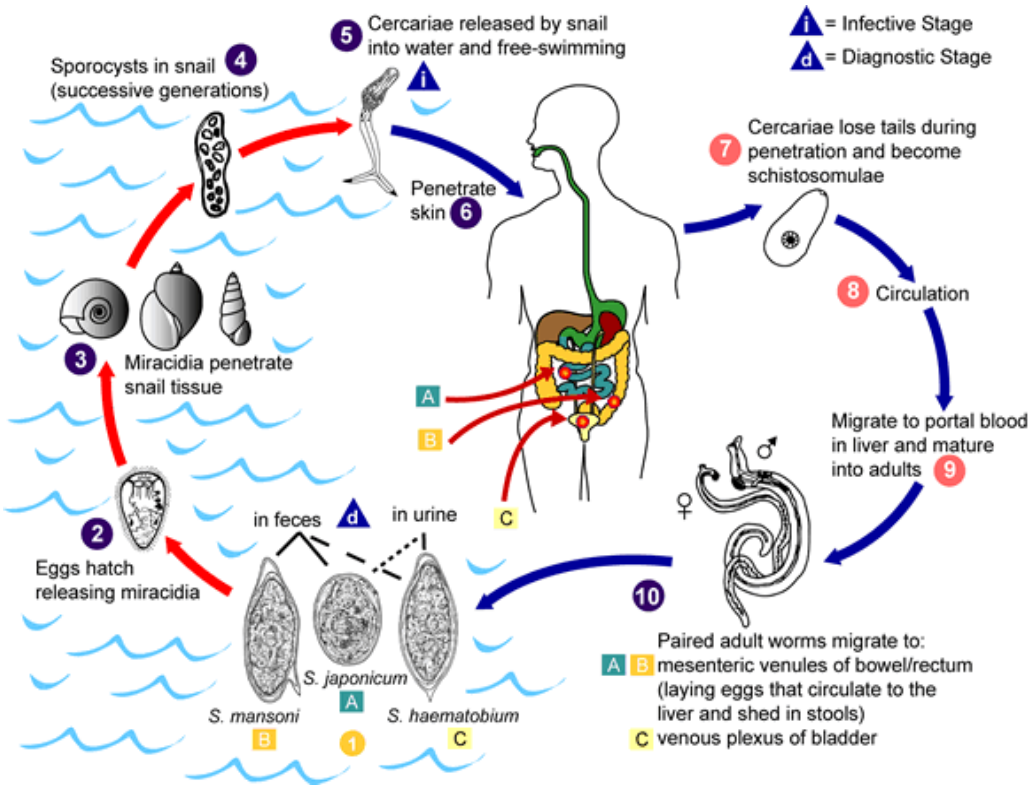


Figure 1: The schistosome life cycle, United States of America Centers for Disease Control and Prevention (CDC 2011).

When a schistosome egg contacts fresh water, it hatches, releasing a miracidia that searches for an appropriate freshwater snail host; in the case of *S. haematobium*, the preferred snail genus is *Bulinus*. The miracidia enters the tissue of the snail, where asexual multiplication of sporocysts takes place over the course of 4 to 6 weeks. The life stage that emerges from the snail is infective to humans and is called the cercariae. Up to 1,500 cercariae can be shed per day by a single snail; this shedding process often takes place in the middle of the day and can occur for up to 18 days (Coon 2005). Once released into a freshwater environment, cercariae have up to 72 hours to locate an appropriate human host or they will die (Gryseels et al. 2006). There are no reservoir hosts of *S. haematobium* (WHO 1993). If a host is found, cercariae will enter the human body via

the skin, lose their forked tails, and become schistosomulae. This larval stage travels through the cardiovascular system, and within about 4 days, it enters the lungs. The larvae spend approximately one week in the lungs and then migrate to the portal veins of the liver where they mature and form adult male-female pairs. Finally, the permanently coupled worm pairs migrate to their final destination, which, in the case of *S. haematobium*, is the venous plexus of the bladder (Coon 2005).

S. haematobium Eggs

Eggs produced by adult worms and excreted from the host live for up to 1 week (Gryseels et al. 2006). Saathoff et al. (2004) are careful to point out that not all eggs excreted by individuals are viable, and not all individuals with eggs in their urine have active infections. Non-viable eggs are often cloudy in appearance when viewed under the light microscope at 40x (K. M. Bosompem, personal communication). Some eggs trapped in tissue may find their way to the lumen of the bladder after adult worms have been successfully killed by praziquantel, but this is probably not common (Saathoff et al. 2004).

Intermediate Snail Host

Bulinus snails are the preferred, but not obligatory, host of *S. haematobium* miracidia. This type of snail can adapt readily to fluctuating water levels (Steinmann et al. 2006), which is not the case for *Biomphalaria* snails, the intermediate hosts of *S. mansoni*. One implication of these characteristics is that urogenital schistosomiasis is more likely than intestinal schistosomiasis to be found near dams and irrigation systems with variable water levels. Snails do not usually live in moving water with velocities greater than 30 cm/sec (Tucker 1983). Naturally occurring,

slowly-moving water is found where watersheds are gently sloped, but may also be created when rivers and streams are purposefully diverted for irrigation, mining, and other types of 'development' projects, or when rivers are dammed and in-stream flows are altered. In natural tropical settings, habitats may disappear during the dry season, sending the mollusks burrowing into the mud, where they cease breeding. Under such conditions, cercarial shedding is impossible and schistosomiasis transmission temporarily ceases. Irrigation schemes and dams that create perpetual water sources where seasonal sources were formerly present often create year-round snail habitats and year-round schistosomiasis transmission (Tucker 1983).

Conditions for the existence of natural populations of *Bulinus globosus* snails are present in much of Ghana, but snail populations were seen to increase around the vicinity of Lake Volta following construction of the Akosombo Dam in 1964 and the filling of Lake Volta by 1968. Lake Volta has 5,000 km of shoreline. An increase in the populations of *Bulinus truncatus* was also observed around the Lake Volta region. Lake Volta reaches its highest levels around October, following the second rainy season of the year in Ghana. It has been observed that human populations are located closest to snail populations at this time of year and that human-water contact is also most frequent at this time (Doumenge et al. 1987).

Risk of Schistosome Infection

Schistosome infections are focally distributed in a population (Gryseels et al. 2006). It is estimated that 80 percent of schistosomes live in 20 percent of human carriers (Anderson and May 1991 as cited in Gordon 2006). Although it is known that infection is heterogeneously distributed, "…analysis and presentation of data at the district, provincial, regional or national levels may not reflect the importance of focal changes. All national programmes must face the

challenge of identifying problem areas and giving priority to action within those areas" (WHO 1993).

The WHO (2002) defines communities at high risk of schistosomiasis as those in which prevalence is greater than 50% among 6- to 15-year-olds. Moderate risk communities are those in which prevalence is between 10 and 50% in the same age group, and in low risk communities, less than 10% of 6- to 15-year olds are positive. The WHO does not specify the method to be used in assessing prevalence or the number of times an individual is to be screened.

Cercariae-containing water must contact human skin in order for infection to occur, but it is common for an individual to be infected by multiple cercariae on one or more occasions over time. Momentarily ignoring the possibility of acquired immunity, the specific worm burden of an individual is directly proportional to the frequency and duration of contact with cercariae-containing water (Clennon et al. 2004). Often, poor adolescents who live in rural parts of the tropics and contact contaminated water frequently and for relatively long durations are a high-risk group, but clearly not the only group at risk. Observational studies can be carried out to determine the age and sex of high-risk individuals and to characterize patterns of water use and water contamination in a community (Kvalsvig and Schutte 1986, Useh and Ejezie 1999). Once risk patterns are known, the underlying issues, such as low socioeconomic status and lack of access to water/sanitation infrastructure, should be addressed as part of a strategy to achieve control of schistosome transmission (Bruun and Aagaard-Hansen 2005).

Because water contact is necessary for infection, Tucker (1983) believes it is the most important variable to consider in terms of transmission control, but other factors must be considered. For example, it is important to understand the reasons for continued use of contaminated water, the means by which surface water becomes contaminated with excreta, the

level of disease awareness among people in endemic areas, social aspects of water contact, seasonal patterns of infection, and population segments most at risk (Husting 1970).

Water Contact Behavior

Water contact behavior has been examined by a number of researchers with varying results. Clothes washing, domestic water collection, swimming, fishing, bathing, and recreation have all been identified as risk factors for schistosome infection (Hammad et al. 1997, Handzel et al. 2003). Handzel et al. (2003) found that self-reported quantity of water contact at Lake Victoria was significantly associated with *S. mansoni* infection and Stothard et al. (2009) found that water contact and water contamination behaviors were strongly associated with *S. haematobium* infection on Zanzibar. Hammad et al. (1997) showed that individuals who self-reported recreational contact (swimming or bathing) with contaminated water were 1.5 times a likely as their peers to be infected with *S. haematobium*. In contrast, Satayathum et al. (2006) report that observed water contact in Kenya did not correlate with infection or reinfection with *S. haematobium* during a nine year period. Pereira et al. (2010) found that lack of access to safe water, the presence of poverty, lack of education, and crowded households were all risk factors for *S. mansoni* infection in Brazilian communities, but the frequency of contact with unsafe water sources was of marginal significance, possibly because of the confounding effects of acquired immunity (Pereira et al. 2010). Data presented by Hammad et al. (1997) also suggest the existence of acquired immunity. Bethony et al. (2001) note that the "the paradigm of exposure to potentially infective water and number of eggs excreted [may be] too simple."

Schistosome infection data can be coupled with knowledge of water contact patterns to contribute to a fuller understanding of transmission dynamics. People who use contaminated

water sources may do so for a number of reasons, one of which is because no other water is available (Tucker 1983). At a study site in rural Brazil, local streams in use for domestic, recreational, and occupational activities were contaminated by flush-pour latrines, but no other water sources were present (Gazzinelli et al. 2001). Even when an improved water source is made available in a rural area, people may continue to use contaminated water. In some cases, this may be because the improved water source does not offer residents the possibility of meeting their recreational, occupational, or other needs (Mafiana et al. 2003, Walker and Walker 1978). Alternatively, the improved water source may be expensive or distant, or there may be misunderstandings about the potential benefits of using an improved water source. In Kenya, some people prefer to use surface water because of the hardness of groundwater (Clennon et al. 2004).

Underestimation of water contact may occur if contact takes place away from a centralized location within a town or village (Friedman et al. 2001). In some cases, water for domestic use is collected from surface waters, brought home, and used by family members who do not themselves visit cercariae-infested waters. Some of these individuals are infants who are bathed in river or lake water by family members unaware of infection risk (Stothard and Gabrielli 2007, Bosompem et al. 2004). In one study, people participating in a focus group could identify red urine as a symptom of schistosomiasis but did not necessarily know that schistosome infections are transmitted through water contact (Mafiana et al. 2003).

To characterize water-contact patterns in a community, one might wish to track: types of water contact (ex. recreational, domestic water collection, bathing); time of day and duration of contact; and age, sex, and occupation of individuals contacting cercariae-contaminated water

(Kvalsvig and Schutte 1986). Stothard et al. (2009) calculated a 'water contact score' based on the self-reported water-related activities of schoolchildren in Zanzibar, Tanzania.

Mafiana et al. (2003) determined normal water contact patterns via focus group discussions, which is a form of self-reporting. Gazzinelli et al. (2001) used self-reporting and direct observation of behavior and found that when water contact sites were near town, there were no significant differences between self-reported and directly observed contacts. However, at sites far from town, such as in the bush or at farm, agreement between the two methods was significantly different, with direct observation tending to underreport water contact rates because the sites were physically removed from the locations where observing researchers were located. Men tended to fulfill different labor roles than women, and because they typically worked and recreated farther from town than did women, they were particularly likely to have their water contacts underreported by observers. The research team suggested that direct observation can be used in the field to develop community-specific questionnaires that will enhance the likelihood of accurate self-reported results (Gazzinelli et al. 2001).

Friedman et al. (2001) interviewed 89 of 91 individuals living in a small community in Brazil. The interviews related to water contact activities; point-prevalence of *S. mansoni* infection in the town was 49%. Interviews were calibrated against direct observation studies performed during 14 days at each of three water contact sites during the hours 7:30 and 17:00. A burn chart (commonly used to describe the body surface area burned on burn patients) was used to estimate the body surface area exposed to contaminated water. Interviews involved the use of a questionnaire with questions about: frequency of water contact, the number of times 11 specific activities were conducted (ex. washing dishes, swimming, bathing, etc.), and duration of each contact. The duration of contact was not reported accurately and so was not asked after the first

set of questionnaires. The questionnaire was re-administered 6 weeks later and the self-reporting was found to be reliable. Correlation between observed and self-reported contacts was analyzed via Spearman rank correlation. 51% of contacts with water were made by only 13% of the population (11 individuals). Boys were never observed to wash clothes or dishes, while girls did so frequently. Boys were much more likely to be observed recreating than were girls (0.53 versus 0.08 times per week, $p = 0.02$). The mean observed exposure to surface water was higher for girls than boys (922 seconds versus 451 seconds, $p = 0.03$) and higher for children under 16 years than for older individuals (820 seconds versus 348 seconds, $p = 0.02$). Overall, the authors concluded that the self-reported frequency of water contact, when supplemented by estimates of the average duration of contact, correlated significantly with observed exposure. In general, individuals who participated in the study tended to report many more water contacts than were observed, suggesting that contacts also took place away from observation sites.

Useh and Ejezie 1999 studied individuals in Adim, a Nigerian community. In 1991, 44% of children in Adim (by 10 ml urine filtration through Whatman No. 1 filter paper) were infected with *S. haematobium*. Adim had neither piped water nor sanitation and no schistosomiasis control programs had been implemented. For their water needs, people used 4 of 12 streams heavily, all of which are located 50 to 150 meters from the town center. As part of the study, behavior at the 4 heavily used streams was observed from 6:00 to 18:00 on Tuesdays, Thursdays, Saturdays and Sundays for one year (Feb 1993 – Jan 1994). Observers recorded age, sex, type of contact, and duration of contact for individuals using the streams. Knowledge, attitudes, and practices (KAP) questionnaires were administered to schoolchildren by their teachers and to illiterate members of households by literate members. One or more urine samples (further details not provided by the authors) were collected from individuals observed contacting surface water.

However, infection data were highly aggregated so that it was not possible to correlate infection status with KAP data or with willingness to alter behavior. Useh and Ejezie (1999) found that: type and duration of water contact were age-dependent; peak prevalence of *S. haematobium* infection was seen among children aged 10 – 14 years; fishing was the most common water activity; bathing and swimming were only seen at one site and accounted for 20% of all contacts; peak bathing/swimming contact as a percentage of total contact was highest among 5 – 9 year-olds; males made more contacts (1,365 vs. 907) and spent more overall time at streams than did females (31,082 min vs. 6,143 min); infection prevalence was 53% in males and 46% in females; and that intensities of infection in males and females were similar. Linear regression showed that infection intensity was more strongly correlated with the number of water contacts per person ($r = 0.97$) than with the duration of the water contacts ($r = 0.77$). After the study, an education campaign was implemented in the community, but the details were not published. Children were also "restrained from bathing/swimming" at the stream considered to be the highest transmission site and community members were discouraged from contacting the water. Given that *only* individuals who contact water were screened for *S. haematobium* in this study, it is interesting that not everyone was infected. This is especially surprising in the 5 to 9 year old age group, as acquired immunity is unlikely. This was not discussed by Useh and Ejezie.

Preliminary data about water contact patterns should be collected before the implementation of a public health intervention designed to address water use. To argue for intervention effectiveness, it is critical to document that people have access to previously unavailable water resources and that behavior has changed (Esrey et al. 1991). The types of preliminary data to collect depend on study objectives. For a study assessing risk factors for schistosomiasis mansoni, Gazzinelli et al. (2001) collected information about the following:

common water-related activities, frequency, and duration of activities, and body percentage exposed to water during activities.

As might be expected, the presence of an observer recording water contacts tends to alter normal behavior and, if the recall of a subject is poor or the time-step in between action and recall is long, surveys can contain inaccurate results (WHO 1979 and Sama and Ratard 1994 in Gazzinelli et al. 2001, Friedman et al. 2001). Direct observation should be carried out as discretely as possible. Gazzinelli et al. (2001) asked a team of observers to work half-day shifts (7:00am to 12:30pm or 12:30pm to 5:30pm) and to record the name, identification number, house number, time spent in the water, and percent of body immersed in the water for each study participant. This information was collected for several weeks during two different time intervals: the rainy season and the dry season. The team also administered a survey of 36 questions, requesting information about the "frequency, type, and location" of normal water contact. It is not clear whether subjects were verbally interviewed or were asked to write responses to survey questions themselves (Gazzinelli et al. 2001). And finally, geographic information system (GIS) and remote sensing tools are now being used to map the distribution of populations affected by schistosomiasis (Clennon et al. 2004, Utzinger et al. 2003).

Irrigation, Dams, and Impoundments

Schistosomiasis is sometimes called a 'disease of development' because the parasite's intermediate host, the snail, is commonly found in slow-moving waters (the preferred habitat) that result from water development activities. Dams, irrigation projects, and mining impoundments are typical ways in which humans alter natural hydrology, and in doing so, create conditions that are ideal for the propagation of schistosomiasis (Chitsulo et al. 2000, Dianou et

al. 2003, WHO 1993, WHO 2006). In the West African countries of Côte d'Ivoire, Ghana, and Nigeria, the construction of large dams preceded an increase in the prevalence of urogenital schistosomiasis (Steinmann et al. 2006, WHO 1993). In 1983, Tucker held that irrigation tended to increase the burden of schistosomiasis because poorly maintained irrigation ditches foster vegetative growth which, in tropical areas, attracts snail species. Polderman (1984) found that the prevalence of schistosomiasis mansoni increased drastically in several rural towns in The Democratic Republic of Congo where tin mining was introduced. Briefly, man-made lakes and canals were created to divert water to the tin mines. These water bodies passed through mining towns and became sites of intestinal schistosomiasis transmission (Polderman 1984).

Provision of safe water and sanitation should be provided as part of water resources development, where such development might increase the risk of schistosomiasis. Safe water and sanitation have benefits that extend beyond prevention and control of schistosomiasis (WHO 1993).

Recreational Water Contact

Use of contaminated surface water for recreation, bathing, and washing are potential risk factors for contracting schistosomiasis (Danso-Appiah et al. 2004, Katsivo et al. 1993b, Kvalsvig and Schutte 1986, Lima e Costa et al. 1987, Mafiana et al. 2003, Ndyomugyenyi and Minjas 2001, Opara et al. 2007, Pitchford 1958, Raso et al. 2005). School-aged children usually have higher schistosome exposure levels than other age groups because of their tendency to recreate in water and their typical water collection duties (Katsivo et al. 1993b, Ndyomugyenyi and Minjas 2001, Olsen 1998, as cited in Gordon 2006). These same children may also urinate and defecate near community water sources, making them both carriers and transmitters of infection (Pitchford

1958). Of all of the aforementioned schistosomiasis risk factors, recreational exposure deserves substantial attention for a number of reasons. Recreation often takes place around midday (Tucker 1983), although reasons for this vary by location. Midday is the time of peak egg excretion into the urine, and also the time of peak cercarial shedding by infected snails (Tucker 1983). Therefore, by collecting water, bathing, or recreating at midday, school-aged children are at high risk for contracting and spreading the parasite because of the biology of the snail, the cercariae, and schistosome eggs. Finally, recreational use of water often involves a relatively large percentage of skin exposure to water for prolonged periods of time (Ndyomugyenyi and Minjas 2001).

In a study conducted by Lima e Costa et al. (1987) in Comercinho in southeastern Brazil, 1017 individuals reported water contact, representing 84% of the population. Of these contacts, three quarters were for "household activities or bathing" and 21% were for recreation. The recreational use of river water tended to be highest in the age group 0 to 14 years. 90.2% (n = 1329) of this population submitted stool samples for *S. mansoni* testing and 70.4% were egg-positive. 92.2% of people who reported water contact (n = 921) for any reason were infected with *S. mansoni*, while 65.7% of those who did not report water contact (n = 236) had eggs (Lima e Costa et al. 1987). It is unclear why individuals who did not report water contact were egg-positive; possibly, true water contact patterns were incorrectly recalled, reported, or categorized; perhaps people in Comercinho did not consider the use of hauled river water in the home as 'water contact'; or perhaps a temporal discrepancy exists, in which eggs come from old infections long after water contact has ceased.

Recreational water contact by pre-primary school children was reported on by Opara et al. (2007). It was observed that the population of Nigerian children studied spent time

swimming, bathing, and washing alone at surface water sites after the age of 3 years. The prevalence of urogenital schistosomiasis increased among children enrolled in the study (n = 126) from the age of 2 years to 5 years, but no infections were found in children under 2 years (Opara et al. 2007). In a study of Tanzanian schoolchildren aged 5 to 19 years, Ndyomugyenyi and Minjas (2001) found that the prevalence of urogenital schistosomiasis was higher among children who reported recreational contact (swimming, bathing, playing) with contaminated water than among children who denied using surface water for the same activities. Individuals in Kenyan communities preferred surface water over borehole water and open wells because of bacterial contamination of wells, hardness of groundwater, and because recreation and bathing were possible only at surface water bodies (Clennon et al. 2006).

Individuals of all ages were observed as they contacted surface water in a Nigerian study of urogenital schistosomiasis. Although the population was observed to carry out 4 main water-related tasks (fishing, fetching water, swimming/bathing, and laundry/utensil washing), fetching water and swimming/bathing were clearly the activities placing individuals at the highest risk of infection based on the total amount of water contact time and the frequency with which these activities were carried out (Oladejo and Ofoezie 2006). These water contact activities were sex-related, with boys found to carry out 67.3% of the 153 observed swimming activities and girls found to carry out 68.4% of the 212 fetching activities.

No literature has been found in which researchers have studied the effect of infrastructure on recreational water contact. A number of papers describe schistosomiasis and recreation (Gazzinelli et al. 2001, Kloos et al. 1986, Lima e Costa et al. 1987, Mafiana et al. 2003, Ndyomugyenyi and Minjas 2001, Opara et al. 2007), but only one (Tucker 1983) specifically calls for interventions to be designed that address recreational exposure. A larger group of papers

discuss the need for some combination of water facilities in communities, sanitation, chemotherapy, snail control, and education, but do not mention recreational water use (Chitsulo et al. 2000, Clennon et al. 2004, Engels et al. 2002, Kabatereine et al. 2005, King et al. 2004, King et al. 2006, Lancet 2004, Nsowah-Nuamah et al. 2004, Polderman 1984, Utzinger et al. 2003, WHO 1993, WHO 2000).

Occupational Water Contact

Occupation may place a person at risk for schistosomiasis if the work causes a person to contact cercariae-carrying water. Fishing and working with irrigated agriculture systems have been identified as particularly common occupational exposure routes (Watts 2005). Communities in a Nigerian study relying on a local reservoir for fishing livelihoods frequently contacted contaminated water. This study found that most people did not believe that an alternative water source would prevent schistosomiasis. Instead, villagers wanted a clinic stocked with anti-schistosomiasis drugs (Mafiana et al. 2003). Other occupations associated with schistosomiasis are: fishing (Farooq et al. 1966 as cited in Gordon 2006), washing cars (Karanja et al. 1997 as cited in Gordon 2006), irrigated agriculture (Watts et al. 1998 as cited in Gordon 2006), and farming (Pereira et al. 2010).

School Attendance

Populations of children enrolled in school have been found to have lower prevalences of schistosomiasis than do children who do not attend school (Watts 2005). This is especially true for unenrolled girl children as compared with girl children who attend school (Watts 2005). In some areas where school attendance is low, children who do not attend school may be at higher

risk of heavy infection than their peers, while being simultaneously more difficult to reach with mass praziquantel administration programs (WHO 1993). In Egypt, lower education status and lack of school enrolment were associated with an increased risk of *S. haematobium* infection; this is likely because of school-based diagnosis and treatment programs (Hammad et al. 1997).

Individuals between 0 and 20 Years of Age

Adolescents between the ages 5 and 15 years typically have the highest prevalence and intensity of infection (WHO 1993). In a community-wide study conducted by Savioli et al. (1990) in Tanzania, children aged 15 to 19 years had the highest prevalence of urogenital schistosomiasis, while children aged 10 to 14 years had the highest prevalence of heavy infection (≥ 50 eggs/10ml urine). Among 4,168 people from a rural community in Tanzania, Bradley and McCullough (1973) found that the highest prevalence of disease was in individuals aged 10 to 14 years (85.3%) and 15 to 20 years (81.8%), as compared with prevalences in other age groups (age range 2 to 90 years, prevalence range 41.2% to 69.9%). King et al. (2004) found a peak in infection prevalence (78%) among adolescents 10 to 14 years old in rural Kenya. Hatz et al. (1998) found that among a population of schoolchildren in Tanzania (n = 533, age range 7 to 17 years), urinary tract pathology as assessed by ultrasound increased with each 1-year increase in age (Relative Risk (RR) = 1.17, CI = 1.064 – 1.292). Oladejo and Ofoezie (2006) found that in a population of Nigerian schoolchildren (n = 320), prevalence of disease and intensity of infection (as assessed by a single egg count) peaked among 12 and 13 year-old children.

The existence of an age-related schistosomiasis infection pattern is relevant in an African context because of the relative youth of the populations of many African nations. In countries endemic for schistosomiasis, roughly 30 to 35% of the total population is school-age children,

and thus, based on typical behavior characteristics, a large percentage of many African populations are at risk of contracting schistosomiasis. For formulas to estimate community prevalence based on infection levels in schoolchildren, see Guyatt et al. (1999). Van der Werf and de Vlas (2004) found in a meta-analysis of 19 studies that presented data from 16 schools and 16 communities that bladder pathology was 35% lower in communities than in schools.

Although the literature usually focuses on school-age children aged 6 to 15 years old (Stothard and Gabrielli 2007, Mafiana et al. 2003), children aged five years and under can also be exposed to schistosomes and may be both carriers and transmitters of the infection (Bosompem et al. 2004, Kabatereine et al. 2005, Opara et al. 2007, Stothard and Gabrielli 2007). For example, Nigerian children as young as 4 years old reported daily use of a local reservoir for recreation and domestic chores, and infection prevalence in these children (n = 209) was found to be 71.8% (Mafiana et al. 2003). Other studies of infant schistosomiasis have found that children and infants as young as 4 months old swim in contaminated water and are infected with *S. haematobium* (Stothard and Gabrielli 2007, Bosompem et al. 2004). In the Cross River Basin in Nigeria, 126 children under 5 years of age were tested for *S. haematobium* eggs and 19.8% (n = 25) were positive (Opara et al. 2007). In the same population, 32.5% of children presented with hematuria (Opara et al. 2007). Kabatereine et al. (2005) note that young children can be passively exposed to schistosomes based on the water contact patterns of their caregivers. Any schistosomiasis intervention should account for water use by infants and children, whether the use occurs because of their own water source preferences or the preferences of others. Mafiana et al. (2003) note that although most infections in young children are light, they should still be taken seriously because the immune system develops throughout infancy and childhood and an

infant's energy expenditures should be focused on growth and development, not on fighting chronic worm infections (Goodman et al. 2007).

Water Contact Differences between Males and Females

The relationship between sex and schistosomiasis infection is highly community-dependent (Gordon 2006). In a study conducted in rural Brazil, women averaged almost 3 times as many contacts with cercariae-contaminated waters as men, but this difference was only present in people over 20 years of age (Gazzinelli et al. 2001). Despite the differences in water contact patterns among older age cohorts, the researchers found no differences in overall prevalence of *schistosomiasis mansoni* by gender (Gazzinelli et al. 2001).

Husting (1970) directly observed water contact behavior in Rhodesia (present day Zimbabwe) over the course of 19 months by people of all ages; females averaged 3.93 arrivals per hour (45 minutes average contact time), compared with 0.92 per hour for males (39 minutes average contact time). 70% of all female water contacts involved washing dishes, collecting water, and swimming. Over 60% of male water contacts involved bathing and swimming. It was found that contacts involving domestic chores tended to involve a substantial social component. Women of all ages tended to cluster at water contact sites together. Males typically clustered in groups of other males in the same age range, with the exception of young boys, who arrived and clustered with women and girls (Husting 1970).

Saathoff et al. found that among South African children under nine years of age, girls were much less likely to be infected than boys (37% versus about 60%, respectively). As age increased, however, boys tended to plateau around 80% prevalence while infection prevalence among girls increased to around 90%. These variable prevalence patterns may be due to different

water contact patterns (Saathoff et al. 2004). In Burkina Faso, boys were found to have a higher prevalence of urogenital schistosomiasis than girls in a pre-treatment cohort of 1,727 children (Touré et al. 2008). Boys also had higher mean infection intensities than girls and tended to be re-infected more often than girls, post-treatment (Touré et al. 2008). Ndyomugyenyi and Minjas (2001) found that for the majority of water-contact activities, boy children self-reported more contacts with water than did girl children. Correspondingly, prevalence and intensity of urogenital schistosomiasis was higher among boys (Ndyomugyenyi and Minjas 2001).

Socioeconomic Status

The connection between poverty and schistosomiasis is beginning to receive more attention. It is telling that schistosomiasis has been proposed as an indicator for poverty and other poverty-related diseases (Watts 2005).The rural poor tend to be most at risk of living with chronic schistosomiasis infection (Gryseels et al. 2006, Watts 2005, WHO 2002). Poverty places people at risk for contracting schistosomiasis, and the disease itself is considered by many to be a poverty-promoting condition (WHO 2005).

Characteristics of poor populations include a lack of access to health and education services, lack of access to sufficient water and sanitation infrastructure (Watts 2005, WHO 2002), poor nutrition status, and "limited access to social networks essential to obtain resources and overcome periodic domestic crises" (Watts 2005). In an extensive review of the literature, Gordon (2006) found the following: that the poor in Africa are more likely to self-treat with praziquantel for schistosomiasis than go to the formal health care system; that in comparison with the poor, wealthier Africans tend to use the formal health care system more and are more

likely to obtain praziquantel when ill; and that large-scale differences in socioeconomic status tend to be reflected on the community scale.

Based on these findings, Gordon (2006) argues that poverty-related indicators should be collected at the community level because they are associated with infection risk. Indicators of wealth may include the following, but will almost certainly be community-specific: presence of an iron roof, improved pit latrine, piped water, concrete floors; child's clothes in good condition; child wearing shoes; child owning full school uniform; number of children in family; mother or father having attended post-secondary school; and parental membership on a school committee (Filmer and Prichett 2003 as cited in Gordon 2006). These indicators of wealth can help describe who has access to treatment, and thus clarify which population segments remain underserved (Gordon 2006). Lima e Costa et al. (1987) found a correlation between indicators of poverty and *S. mansoni* infection in a Brazilian town. *S. mansoni* tended to affect people in households headed by manual workers, as compared with people who owned businesses, were retired, or were skilled workers. Prevalence was higher among people who did not have piped water supplies and those who had been born in the town, as compared with residents who had piped water and who had moved to the town from elsewhere (Lima e Costa et al. 1987).

Newcomers to an Endemic Community

Kloetzel (1992) found that individuals who had lived for less than two years in a highly endemic community in Brazil were heavily infected with *S. mansoni*. He recommends treating all new residents in endemic areas, preferably with the support of community-based organizations (Kloetzel 1992).

Previous Infection Status

Previous infection with schistosomes is a complex variable. It could indicate behavior that increases risk of reinfection, but could also be associated with a relatively high likelihood of recent treatment with praziquantel (Hammad et al. 1997). Finally, previous infection may be associated with acquired immunity. Via multivariate logistic regression analysis, Rudge et al. (2008) found that prior treatment for schistosomiasis was negatively associated with heavy infection (OR: 0.031, $p = 0.018$).

Analysis of Risk Factors via Logistic Regression

Binary logistic regression can be used to predict the likelihood of an event such as the presence of disease or the presence of infection, given specific values of explanatory variables (Bagley et al. 2001, Bewick et al. 2005). The ability to accurately predict schistosome infection status without filtering a urine sample would be a valuable addition to the public health screening 'toolbox' in schistosomiasis-endemic settings; urine filtration is time consuming, labor-intensive, expensive, and requires skilled personnel and specialized equipment. Accurate predictions of infection would also reduce the likelihood of medicating schistosome-negative children. However, it is important to note that models predicting the likelihood of infection with schistosomes in one community may not accurately predict infection elsewhere, given that schistosomiasis is location-specific and depends on behavior and other host factors. In addition to predicting infection status based on characteristics and/or tests other than urine filtration, logistic regression models can be used for hypothesis testing and in the statistical assessment of risk factors that influence infection or disease.

In addition to using logistic regression models for prediction, they can be used to assess the "relative contribution" of various predictor variables (also called 'independent variables' or 'covariates') to the dependent variable (the outcome) in the model (Bagley et al. 2001). The coefficients in a final logistic regression model can be interpreted as the statistically significant increase in the odds of an outcome, given a 1.0 unit increase in the value of the corresponding independent variable.

Potential risk factors for contracting *S. haematobium* infection have been evaluated via logistic regression analysis (Nsowah-Nuamah et al. 2001, Hammad et al. 1997, Stothard et al. 2009). Regression analyses have been used to characterize the extent to which risk factors contribute to the probability of schistosomiasis infection among schoolchildren. The binary dependent variable is usually "infection status". Among others, the independent variables may include: age, sex, education level, exposure type (ex. domestic, occupational), previous infection status, season, community size, water contact patterns, previous infection status and presence of an intervention against schistosomiasis. Hammad et al. (1997) used their model to assess the confounding effects of age and gender on risk of exposure and on likelihood of infection. The authors concluded that because age and gender offer little explanatory power, age-acquired immunity to schistosomiasis is likely for the Egyptian population under study. Nsowah-Nuamah et al. (2001) developed a logistic regression model to predict probability of infection among individuals living in the presence or absence of an intervention aimed at curtailing exposure. Two years post-intervention, people living in areas where "active health education" was used for schistosomiasis prevention had 0.15 times the odds (see *Logit Function*) of schistosomiasis as did people living in areas without health education. "Active health education" involved the construction of wells and latrines and the removal of weeds from surface water bodies. Stothard

et al. (2009) found that prior infection with schistosomes indicated that individuals were significantly more likely than their peers to be reinfected after treatment.

Hatz et al. (1998) also used logistic regression to characterize the effect of age on bladder pathology associated with urogenital schistosomiasis, but did not provide information about model development, model assessment, or model results.

Odds and Odds Ratios

Logistic regression is used to predict the probability that an outcome will happen (Bewick et al. 2005). A probability can take on any value between 0 and 1. Linear regression is appropriate for modeling continuous outcome variables, but not for modeling probabilities. Instead, logistic regression is used. The dependent variable represents the natural log of the odds of the outcome (Bewick et al. 2005). 'Odds' are defined as follows:

$$Odds = \left(\frac{p_i}{1 - p_i} \right), \text{ where}$$

p_i = the probability of an event happening

Odds differ from an 'Odds Ratio'. An 'odds ratio' is defined as follows:

$$Odds\ Ratio = \left(\frac{\text{odds of experiencing an outcome given exposure}}{\text{odds of experiencing an outcome given no exposure}} \right)$$

Essentially, the odds ratio quantifies the expected increase in risk (ex. contracting a communicable disease) under specific conditions (ex. being female, being older than 40 years, etc.).

Logit Function

In logistic regression, the dependent variable is also called the 'logit function', and is defined as follows:

$$Logit(p_i) = \ln\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \beta_1 x_{1,i} + \beta_k x_{k,i}$$

The logit function is useful because it may take on real values ranging from -∞ to +∞, in contrast to probabilities, which are constrained to real values between 0 and 1 (Hosmer and Lemeshow 1989, Bewick et al. 2005). In addition, the logit function may be continuous (Hosmer and Lemeshow 1989), as opposed to categorical. The distribution of a logistic regression model is binomial, and because of this, statistical analysis programs are typically employed to use iterative techniques to estimate parameters based on a method of maximum likelihood estimation (Bewick et al. 2005, Hosmer and Lemeshow 1989). For a more complete discussion of maximum likelihood estimation and the likelihood function, see Hosmer and Lemeshow (1989, p 8 – 11) and McCullagh and Nelder (1983, as cited by Hosmer and Lemeshow 1989). The errors in a logistic regression model are also binomially distributed. Solving the logit for the probability of an event (p_i), it can be shown that (Bewick et al. 2005, Hosmer and Lemeshow 1989):

$$p_i = \text{probability of an event} = \left(\frac{e^{(\beta_0+\beta_1 x)}}{1+e^{(\beta_0+\beta_1 x)}}\right), \text{ where}$$

β_0 = model parameter

β_1 = model parameter

It has been shown elsewhere (Bewick et al. 2005) that in the case of a model with a single explanatory variable, the odds ratio is equal to ($e^{\beta 1}$). The odds ratio associated with an explanatory variable can be interpreted as the increased risk of experiencing an event, given a 1.0 unit increase in the explanatory variable.

Assessing the Significance of Model Coefficients

Each model coefficient (β_n) must be assessed to determine whether it contributes significantly to the overall logistic regression model. The significance of a coefficient is different from the goodness-of-fit of a model (see *Goodness-of-Fit*) based on the inclusion of the coefficients. A significant coefficient causes a model to fit data better than a model that does not include the coefficient. Hosmer and Lemeshow (1989) describe the idea as: "Compare observed values of the response variable to predicted values obtained from models with and without the variable in question" to determine whether the coefficient causes significant change. Goodness-of-fit is evaluated later.

Interaction between Predictor Variables and Confounding

A logistic regression model that includes more than one predictor variable may contain a "confounder" variable, which is defined by Hosmer and Lemeshow (1989) as a "covariate that is associated with both the outcome variable of interest and a primary independent variable or risk factor." A confounder can obscure the relationship between a predictor variable and the outcome

variable (the logit function), and thus the logistic regression model must account for it explicitly. 'Confounding' and 'interaction' are distinct phenomena. 'Confounding' simply indicates that there is a relationship between a predictor variable (confounder), a different predictor variable, and the outcome variable (the logit function). 'Interaction' indicates that one predictor variable, an "effect modifier", alters the effect of another predictor variable, in terms of the value of the outcome variable (Hosmer and Lemeshow 1989). A 'confounder' may also be an 'effect modifier', but this is not always the case. A logistic regression model that accounts for interaction among predictor variables will contain higher order terms.

Schistosomiasis Control

Control of schistosomiasis should part of the regular focus of the pre-existing primary health care system (WHO 1993). A long-term commitment to control of schistosomiasis is necessary; the appropriate timeline will depend on a country's level of socioeconomic development, but will probably be in the range of 10 to 20 years (WHO 1993). Unfortunately, there is very little effective schistosomiasis control in sub-Saharan Africa. Many of the control efforts that began in the 1980s were funded by external donors, and it was expected that once these efforts were underway, local governments would commit internal resources to continue the programs. This did not happen, possibly because health authorities believed that schistosomiasis was a relatively low national priority (Engels et al. 2002). In countries with small health-related budgets where schistosomiasis is endemic, it is necessary to consider schistosomiasis control in the context of controlling other, more pressing public health problems such as malaria, diarrheal disease, or HIV/AIDS. Control strategies were recently reviewed by King (2010) and are summarized in Table 1.

Pros and cons of different schistosomiasis control strategies.

Intervention	Advantages	Disadvantages	Relative cost	Impact on disease
Drug treatment				
Mass drug delivery	High initial uptake. Wider advocacy and community mobilization. Rapid impact	Reduced adherence over time. Incomplete cure of heavy infections. Transmission continues unabated in worst areas. Risk of drug resistance over time	+++	Partial control of early and late morbidities. Indefinite need for re-treatment
Age-targeted drug delivery	Structured framework for delivery where community census data are limited. Rapid impact	Often poor coverage for children who are not in school. Incomplete cures. Transmission continues. Risk of drug resistance over time	++	Partial control of early and late morbidities. Indefinite need for re-treatment
Symptomatic treatment	Limited cost. Focus on controlling or preventing late morbidity	Symptom driven. In practice, limited access and inadequate diagnostics mean that treatment is rarely given	+	Late outcomes improved but not prevented. These are often irreversible
Health education	Important advocacy for control, shift in behavioural risk. Collateral benefits for other disease prevention	Time and teacher dependent. Cultural context may determine acceptance	+	Health education frequently has limited impact when used alone due to lack of choices in water use
Snail control				
Areawide	Limited need for community mobilization	Cost. Cross-species toxicity. Requires high tech personnel input	+++++	Gradual reductions in human prevalence and disease
Focal	Lower cost and side-effects than areawide	May miss important transmission points	+++	Gradual reductions in human prevalence and disease
Habitat modification	Greater initial costs. Need for land management planning and community mobilization	Indirect effects of local changes in ecology	++++	Single interventions may have only minimal impact on area transmission
Water supply and sanitation				
Latrines/sanitation	Impact on multiple infections	Requires significant changes in human behaviour	+++	Performed alone, may have only limited impact on schistosomiasis due its to leveraged, networked transmission
Basic well or piped water	Impact on multiple infections	Technology dependent, uptake not universal	+++	Can reduce risk of transmission and slowly reduce morbidity in some areas
Water plus sanitation	Impact on multiple infections	(As above)	++++	Can reduce risk of transmission and slowly reduce morbidity in most areas
Water/sanitation plus alternative laundry, bathing, recreation	Impact on multiple infections	Requires further significant changes in many daily activities and human behaviour	+++++	Can eliminate transmission and slowly eliminate morbidity in most areas. The definitive solution to transmission if no zoonotic reservoir exists

Table 1: "Pros and cons of different schistosomiasis control strategies" (taken directly from King 2010).

Appropriate control strategies are often location-specific, but aspects of parasite-human-environment interaction should be noted during the design process. For example, a single exposure to cercariae-contaminated water is sufficient to cause infection, even when snail densities and the number of infected snails are low (King 2010). One infected individual can heavily contaminate an entire water body for months (King 2010, Kloetzel 1992). Because contaminated water bodies may be used by individuals from various communities, transmission

of *S. haematobium* may continue when control measures are implemented in some communities but not in neighboring ones (King 2010). As Wang et al. (2009) has pointed out, "the process of local transmission is often saturated, and egg contamination must be significantly reduced before it declines (as cited by King 2010). In most countries, morbidity data are lacking and an accurate assessment of schistosomiasis-related morbidity cannot be made. Nevertheless, it is necessary to create a strategy to estimate the effectiveness of control programs (WHO 1993).

In China, control of *S. japonicum* has typically relied on chemotherapy and snail control; in 2004, the Chinese government developed new targets for further reducing the prevalence of *S. japonicum* infection. A strategy of primary prevention was evaluated by Wang et al. (2009) for its ability to meet these targets. The main exposure routes among study participants were fishing, agriculture, swimming, and laundry. Prior to the study, chemotherapy and health education were the main control activities. The primary prevention program evaluated by Wang et al. consisted of three main activities: (a) elimination of cattle (a *S. japonicum* reservoir host); (b) implementation of water and sanitation facilities, mainly in the form of tap water and latrines; and (c) outreach in the form of health education, with a strong focus on the relationship between fecal matter and *S. japonicum* infection. 71% of the funding for this program was provided by the Chinese government; the total cost was US$373,200 for the two interventions communities. Cross-sectional studies of population-level *S. japonicum* infection were carried out for 6 years in the absence and then in the presence of the control programs; these levels of infection were compared with control communities that did not receive the package of intervention activities. The authors found statistically significant declines in infection prevalence among intervention communities, but not among control communities. The percentage of aquatic habitats that contained *S. japonicum*-infected snails also decreased. The authors concluded that removal of

cattle, a reservoir host of *S. japonicum*, was probably the most important control mechanism implemented (Wang et al. 2009). Because *S. haematobium* and *S. mansoni* do not have reservoir hosts, this same control strategy is not applicable, but the concept of integrated control and the idea of identifying main schistosome exposure routes and preferentially addressing these routes is a universal lesson.

Urogenital Schistosomiasis: Morbidity Control

Between 1984 and 1991, the WHO recommended a strategy of morbidity control. In its 1993 report, the WHO promoted additions to the morbidity control strategy in the form of health education as well as the provision of water and sanitation to at-risk populations. As of 2005, morbidity control through regular administration of praziquantel, the drug of choice, was still the preferred schistosomiasis control method (Watts 2005). Once morbidity control is achieved, national health ministries are urged to focus on reducing intensity and prevalence of infection; transmission cessation is the ultimate goal (WHO 1993). The strategy of morbidity control has been called "both feasible and effective" (WHO 1993).

When morbidity control is defined as a reduction in egg excretion, control efforts may be insufficient if they fail to address adequately the problem of widespread 'light' infections in individuals. Individuals excreting few eggs can present with, or be at risk of developing, serious pathology if they are genetically predisposed to high inflammation (King et al. 2006).

Drug Treatment of Schistosomiasis

Praziquantel is the drug of choice to treat all types of schistosome infections (*S. haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. matthei, S. mekongi*), but oxamniquine is also

available to treat *S. mansoni* infections and artemisinin derivatives are effective against larval-stage schistosomes (Cioli 2005). Adult worms are partially sensitive to artemisinin derivatives (Cioli 2005). Praziquantel is only effective against adult schistosomes, and is most effective when given at least 6 to 8 weeks post-infection (Cioli 2005). Treatment of urogenital schistosomiasis is an important step in the prevention of bladder cancer (WHO 1993). Despite the clinical effectiveness of praziquantel, it is not clear whether treatment, vaccine development, or water resources infrastructure will most effectively combat schistosomiasis at the population level (Gordon 2006). It is generally accepted that chemotherapy alone is not an effective, sustainable strategy (Bruun and Aagaard-Hansen 2005, Stothard et al. 2009, Tucker 1983, Utzinger et al. 2003, WHO 1993, WHO 2005).

As recently as 2002, the WHO recommended the use of selective praziquantel administration in areas of low endemicity. The selective treatment would be carried out based on the results of dipstick tests for hematuria. However, dipstick tests may fail to identify a significant number of infected individuals (Kosinski et al. 2011).

Praziquantel Efficacy

Praziquantel usually kills all adult worms in 60% to 90% of those infected and reduces egg counts by 85% to 95% among those who are not cured (WHO 1993, Cioli 2005). However, the methods of assessing infection status and egg count are imprecise and do not provide information about the number of adult worms that are killed (Geary et al. 2010). Praziquantel is usually given at a dose of 40mg/kg or 60mg/kg of bodyweight. It should be administered after a patient has eaten (WHO 1993). Saathoff et al. (2004) used infection intensity to assess efficacy of treatment with praziquantel (40 mg/kg) in a study conducted in a number of primary schools in rural South

Africa. Geometric mean egg reduction and the cure rate of heavy infections (≥ 50 eggs/10ml urine) were both very good (95.3 and 94.1%, respectively) at 3 weeks post-administration of praziquantel. Nsowah-Nuamah et al. (2004) found that praziquantel decreased mean egg count 5 weeks after treatment in 8 Ghanaian communities. Mean egg counts for 3 geographic areas ranged from 201.4 to 271.8 eggs/10ml urine before treatment and from 1.4 to 16.1 eggs/10ml urine after treatment. In Burkina Faso, Touré et al. (2008) concluded that a single mass administration of praziquantel during the dry season reduced prevalence of urogenital schistosomiasis from 59.6% to 6.2% one year later and to 7.7% two years later. Infection intensity was also significantly reduced: before treatment, more than 25% of children in the study were heavily infected (> 50 eggs/10ml urine), whereas one year post treatment, only 0.4% were heavily infected and two years post treatment, less than 2% were heavily infected (Touré et al. 2008).

Perhaps more important than reducing the prevalence and intensity of schistosomiasis, praziquantel is able to reduce clinical morbidity, as characterized by ultrasound. In a study of 244 Tanzanian schoolchildren (age 7 to 17 years), Hatz et al. (1998) found that at baseline, 170 children (75.9%) had urinary tract pathology as assessed by ultrasound. Praziquantel was administered, and pathology was reassessed by ultrasound on six occasions (2, 4, 6, 12, 18, and 24 months) post-treatment. 88% of children presented without lesions within 6 months of praziquantel administration, and by 2-years post-treatment, 96% had presented without lesions. However, the study area was highly endemic for schistosomiasis and 57.1% of the children developed new lesions after having initially resolved the pathology (Hatz et al. 1998), suggesting that while praziquantel is effective, it is not sufficient to control long-term morbidity unless it is repeatedly administered.

Praziquantel significantly improves anemia status in randomized controlled trials (King et al. 2005) and is accepted as a crucial tool in the prevention of severe schistosomiasis morbidity (WHO 1993). As quickly as 6 months post-treatment with praziquantel, schistosomiasis-induced pathology can be reversed (WHO 2006). Schistosomiasis-induced lesions of the bladder and kidneys heal well after treatment with praziquantel (Gryseels et al. 2006), providing strong evidence in support of regular treatment campaigns. In 2001, the World Health Assembly stated that praziquantel should be provided regularly to at least three quarters of children living in high-transmission areas (WHO 2002). In 16 schools in Burkina Faso, mass treatment with praziquantel was sufficient to reduce the prevalence of urogenital schistosomiasis, and to keep prevalence below 8% (from 59.6%) two years after treatment (Touré et al. 2008). However, it is important to note that praziquantel was administered in the dry season, the year in which it was distributed (2004) was uncommonly dry, country-wide drug coverage exceeded 90%, and mass treatment took place during a relatively short period of time. When these conditions are not met – for example, "should drug distribution be interrupted" – infection rates can quickly increase (Touré et al. 2008). At present, widespread drug resistance to praziquantel does not seem likely, but research into alternatives should be undertaken immediately, as reliance on a single compound for treatment is extremely dangerous.

Mechanism of Action and Side-Effects of Praziquantel

Praziquantel is a safe, effective drug that can be administered by non-medical personnel (Engels et al. 2002). It is the drug of choice for treatment of schistosomiasis because it is considered to be relatively inexpensive (cost varies), effective, and side effects are usually minimal (Gryseels et al. 2006, WHO 2002). There is little information available about the absorption of praziquantel

in field settings, particularly when the drug is given with food; more research is needed in this area (Geary et al. 2010).

Praziquantel side effects may include "nausea, vomiting, malaise, and abdominal pain", but in heavy infections (≥ 50 eggs/10ml urine), can be somewhat more severe (Gryseels et al. 2006). Side effects could be minimized if drug manufacturers were to eliminate non-effective isomers from praziquantel pills; at present, the pills contain a mixture of isomers, only one of which is effective against the adult worm (Cioli 2005). In the field, side effects can be minimized by ensuring that praziquantel is not taken on an empty stomach (WHO 2002) and by splitting the dose in half and administering each half a number of hours apart.

No adverse effects on pregnant women or unborn children have been shown (Allen et al. 2002), despite the fact that praziquantel has been used by human populations since 1980. In contrast, schistosomiasis has been shown by a number of research groups to be associated with serious adverse outcomes in pregnancy (Olds 2005). Praziquantel is a category B drug, meaning that it has been assumed to be safe in animal models, but has not been explicitly tested in humans mainly because it is difficult to obtain ethical clearance for such studies. The unfortunate consequence of this difficulty is that many women infected with schistosomes remain untreated for long periods of time, a situation that elevates their risk of anemia and severe long-term morbidity (Olds 2005). Olds (2005) has pointed out that anemia is the leading health problem facing women worldwide: "in developing countries almost half the women are iron deficient." In the opinion of Olds (2005) who wrote the WHO working paper on schistosomiasis during pregnancy, treatment of pregnant women with praziquantel to cure their schistosomiasis is good for the women, but the consequences for their unborn children are not as clear. There is a risk of a high pro-inflammatory response following treatment of schistosomiasis and subsequent rapid

reinfection during pregnancy in highly endemic regions. In such a situation, and especially during the third trimester of pregnancy, the growth of the unborn child could be adversely affected by inflammation (not by praziquantel). Additional studies will need to be carried out before praziquantel can be indisputably recommended for use during pregnancy (Olds 2005).

Praziquantel is an acylated quinoline-pyrazine (Gryseels et al. 2006). It is effective against all schistosome species that parasitize humans and works by increasing muscle contractions in adult schistosomes, which causes the detachment of worms from host tissue; when the dose is sufficiently high, praziquantel reduces a worm's ability to regulate cation levels (Coon 2005). However, the exact mechanism of action of praziquantel remains unknown (Cioli 2005). 80% of a praziquantel dose is excreted from the human body within 24 hours (WHO 1993). Praziquantel is ineffective against larval schistosomes, and efficacy is greatest 6 to 8 weeks post-infection (Cioli 2005). Administration of praziquantel often causes a reduction in schistosomiasis-related morbidity, but in the late stages of disease, severe pathology may not be reversed (WHO 2005).

Praziquantel Administration and Age of Target Population

Although praziquantel is approved for use by children and pregnant women (Gryseels et al. 2006), it is not normally administered to infants and very young children. Several researchers have argued convincingly that the excellent safety and effectiveness of the drug should be weighed against concerns that it has not been substantially tested in children under 4 years old. The risk of pathology from continued disease, coupled with no observed adverse events in the field, suggest that praziquantel may be efficacious in infants and very young children (Bosompem et al. 2004, Stothard and Gabrielli 2007). Moreover, the fact that very young

children (aged 2 to 5 years) have been found to be potential infection transmitters suggests that under-fives should be included in treatment and control programs (Opara et al. 2007). In 2002, the WHO recommended administration of dewormers to preschool children in endemic areas on the grounds that "they are often infected with helminths and it is in this age group that growth faltering begins." Gordon believes that there has not been sufficient study of praziquantel use during pregnancy/breastfeeding to evaluate its safety for the mother and the child during these life stages (Gordon 2006). For information about the policy of treating women and girls regardless of pregnancy status, see WHO 2002, Lancet 2004, WHO 2005, and Hotez et al. 2006.

Administration of praziquantel to very young children can be complicated due to the unpalatable taste of the medicine and the large tablet size. The tablets can be broken into pieces and mixed with flavored syrups to disguise the bitter taste (Stothard and Gabrielli 2007). Others have suggested the introduction of praziquantel syrup for treatment of children younger than five years (Opara et al. 2007).

Teenagers in Ghana, especially males, have been found to seek treatment for schistosomiasis less frequently than other members of the population. This may be due to the cost of treatment in Ghana through the 'cash-and-carry' system and the common practice of teenagers covering the cost of their own health care (Danso-Appiah et al. 2004). Danso-Appiah et al. (2004) pointed out the serious consequences of the lack of access to drugs for teenagers; this age group tends to be more heavily infected than other demographic groups and may also be the least likely to receive treatment for infection, which can contribute to long-term pathology.

To calculate the correct dose of praziquantel per person, the preferred method is to weigh an individual and administer 40mg per kilogram of bodyweight, or to use a proxy for weight in the form of a dose pole developed by the WHO for children over 94cm in height (WHO 2002).

Because of the safety of praziquantel, dosing can be carried out by non-medical personnel such as teachers, care-givers, and community health workers (WHO 2002).

Cost of Praziquantel Treatment

Estimates of the cost of praziquantel treatment vary, depending on whether an individual is assumed to self-treat or whether treatment takes place through a mass administration program. A person who self-treats pays for the retail price of the drug and incurs the expenses associated with traveling to purchase the drug and opportunity time lost. The costs associated with travel and lost time can be substantial if praziquantel is not locally available.

Mass treatment programs target praziquantel distribution to schistosomiasis-endemic areas. Multiple methods have been used to distribute the drug, but these methods are often location-specific. Funding for programs to mass distribute praziquantel often comes from external donations, which may suggest that even when these programs are cost-effective, they may not be locally affordable (Guyatt 2003). This is in contrast to a statement made by a WHO Expert Committee (2002): "Regular treatment is affordable and can be delivered in a sustainable manner through existing channels." In the same publication (WHO 2002), the Expert Committee concedes that "…donor support may still be needed for control programmes, especially school-based or community-based interventions in the poorest areas of developing countries…in many developing countries, drug supply systems are still inadequate to meet essential demands at the peripheral level."

The price of praziquantel in Ghana remains high; in 2009, the average dose of 3 pills cost US$0.50. This price does not include any of the associated costs of transportation of the drug and personnel, salaries for those who distribute the drug, or administrative costs. Moreover, the drug

was scarce in Accra, Ghana in July and August of 2009 and six pharmacies had to be visited by the author before the drug was found to be available.

In a mass distribution program, if each person is to be treated based on the results of tests for blood and/or eggs in their urine, the costs associated with testing and drug distribution can be much higher than simply the price of praziquantel (Guyatt et al. 1994). Guyatt (2003) and Guyatt et al. (1994) discuss some of the costs that ought to be, but are not typically included in estimates of praziquantel administration. The main categories enumerated by Guyatt (2003) are: the "opportunity cost of using existing personnel", where personnel are health care workers, schoolteachers, administrative assistants, laboratory workers, or others; consumables, such as forms and stationary; training allowances; salaries; insurance and shipping of drugs to distribution locations; 10% drug wastage; and non-consumables, such as dose poles (Guyatt 2003). When these costs were included, Guyatt (2003) calculated that the cost per child treated in the Partnership for Child Development program in Tanzania was $US 0.625. Additional estimates of the costs associated with various control programs are discussed in detail by Guyatt et al. (1994). However, Guyatt et al. estimated costs based on what are now known to be underestimates of the sensitivity and overestimates of the specificity of a reagent strip test for hematuria, and so the estimates may not be accurate.

To determine who will receive praziquantel, it is sometimes desirable to ask all members of a target population to either self-report macrohematuria or to submit a urine sample for testing with reagent strips that detect microhematuria. Only those who are positive receive praziquantel, which can be fiscally attractive when testing is inexpensive and medication is preserved for later use (Guyatt 2003), but this approach may fail to treat people who are asymptomatic true positives. Presumptive treatment is sometimes used (Guyatt 2003) in endemic areas, and in such

cases, the cost of urine testing may be reduced, compared with testing every individual prior to praziquantel administration. The decision to presumptively treat is usually based on the results of a prevalence survey (self-reported macrohematuria, urine dipstick test for microhematuria, or urine filtration test for schistosome eggs) among a sample of the target population.

Miguel and Kremer (2004) found that when people were asked to partially pay for treatment, use of the drug dropped drastically. The June 2008 retail price in Accra, Ghana was approximately US$0.20 per 600mg pill when praziquantel was bought in bulk (1,500 tablets). On average, a child requires 3 pills for a 40mg/kg bodyweight dose, meaning that the average cost per dose in Ghana was US$0.60. In Uganda, Kabatereine et al. (2005) report that the cost of a single praziquantel pill was US$0.07 in 2005. Writing for the WHO, Cioli (2005) estimated that the cost of praziquantel is approximately US$0.24 per treatment. Kloos et al. (1986) found that in a community in Kenya, traditional medicines are sometimes preferred over "modern" medicines to cure intestinal problems and schistosomiasis mansoni. The main reason for the preference appears to be the high cost of anthelminthics, praziquantel, and other medicines, such as antibiotics. The population studied was aware of the efficacy of conventional treatments, and traditional healers sometimes referred patients to local hospitals and clinics to purchase these medicines. It appears that high cost sometimes prevents the poor from obtaining effective treatment for intestinal illnesses (Kloos et al. 1986).

Mass Administration of Praziquantel

Praziquantel can be safely administered to uninfected individuals (WHO 2003a). Treatment with praziquantel once per year in endemic communities is considered sufficient by the WHO (2003a). The dose pole, where treatment is administered based on height as a proxy for weight, is

commonly used in field settings (WHO 2003a). The WHO (2003a) recommends that treatment be offered to all children in endemic communities and, to contain costs, the children should not first be screened.

Mass administration of praziquantel is beneficial to individuals who receive treatment and to communities where the human reservoir of schistosomiasis is correspondingly reduced (McGarvey 2005, Wilkins et al. 2007). Provision of praziquantel to affected populations is not only beneficial, but is also required by the World Health Assembly resolution WHA54.19. The resolution states that by 2010, 75% to 100% of school-age children (6 to 15 years) in endemic areas should be offered treatment at regular intervals (WHO 2002). In 2003, the WHO (2003a) stated that all children should be offered praziquantel at least once every two years if any child in school has blood in his or her urine.

In areas of low endemicity, the WHO recommended the use of selective praziquantel administration based on the results of dipstick tests for hematuria (WHO 2002). This policy should be reconsidered, given the low sensitivity of dipstick tests used with lightly-infected patients (Feldmeier et al. 1982).

In the WHO 1993 report, recommendations for the mass administration of praziquantel were somewhat different from those recommended more recently. One treatment strategy in 1993 was as follows: (a) treat an entire population if prevalence of infection among 7 to 14 year-olds is over 50%; (b) treat all children aged 5 to 19 years if the prevalence of infection among 7 to 14 year-olds is between 20 and 50%; and (c) treat only positive children if the prevalence among 7 to 14 year-olds is less than 20% (WHO 1993). The screening method to be used for this type of praziquantel administration was not specified.

A substantial amount of coordination is necessary at the community level for successful mass-administration of praziquantel. Before designing a chemotherapy program, it is important to identify which sectors of a population are most at risk for schistosomiasis, and which type of drug administration program will be most effective (WHO 2003a). Some studies have shown that in communities with low to moderate schistosomiasis prevalence, community-based interventions reach more infected people than school-based interventions. Community-based interventions are also more likely to treat women and girls, which is probably due to poor school enrolment of girls in some locations. A benefit associated with school-based interventions is that fewer uninfected people are treated, as compared with community-based programs; schools tend to have higher infection prevalences than communities (Gordon 2006). At the community level, it is essential that individuals carrying out deworming programs communicate the nature of the program and the expectations during and after treatment, especially with respect to the reporting of mild side effects (WHO 2003a).

In primary schools in South Africa, many children were absent from school during the first round of a treatment program; during the second round, once word had spread of the safety and efficacy of praziquantel, far fewer children were absent on treatment days (Saathoff et al. 2004). In endemic areas, Saathoff et al. (2004) recommended that children be treated annually approximately 3 to 4 weeks after the end of a relatively high transmission season, which may correspond to the hot rainy summer months.

Praziquantel causes few serious side-effects and may be administered to at-risk populations by non-medical personnel (Cioli 2005, WHO 2003a). In Uganda, Ndyomugyenyi and Kabatereine (2003) found that when praziquantel was administered in an integrated control program, drug distributors from the communities were rarely financially compensated (17% of

distributors), but in-kind contributions (ex. meals) were somewhat common (30% of distributors). The drug distributors administered praziquantel from centralized points in communities and by traveling door-to-door (Ndyomugyenyi and Kabatereine 2003).

As part of the national schistosomiasis control program in Uganda, teachers administered praziquantel to children through schools (Kabatereine et al. 2005). The WHO (2003a) recommends training teachers to distribute praziquantel via the dose pole, using height as a proxy for weight, and to keep records of treated children. Treatment through the school system should ideally be accompanied by other programs to improve knowledge of worm diseases and sanitation/hygiene infrastructure (WHO 2003a).

The WHO supports the community-directed distribution of ivermectin and albendazole because the method improves treatment coverage and is considered more sustainable than vertical approaches; the WHO recommends that research be undertaken to determine whether these positive aspects are also seen with praziquantel distribution (Kabatereine et al. 2005).

Repeated mass treatment with praziquantel of children living in endemic areas is recommended for the beneficial effect it has on controlling morbidity later in life (King et al. 1992, Hatz et al. 1998, Frenzel et al. 1999 as cited in Engels et al. 2002, WHO 2005), but a more recent review article states that "the average resident's cumulative life-years of infection might only be reduced from 14.6 to 8.8…after implementation of a drug-based treatment program" (King et al. 2006). This suggests that morbidity control is important at the level of the individual, but that treatment with praziquantel is not a substitute for transmission control in endemic areas. In a study of the effect of praziquantel on pathologic urinary tract lesions, Hatz et al. (1998) found that within 2 years of administration of praziquantel, nearly half of children previously infected with *S. haematobium* "again had severe lesions requiring treatment." These findings of

morbidity reappearance suggest that primary prevention of schistosomiasis is needed in addition to treatment campaigns. The WHO (2005) considers morbidity control a "major step forward" for those affected by chronic disease, but is careful to point out that high reinfection rates are unacceptable and necessitate further action.

Self-Treatment with Praziquantel

In most endemic settings, individuals have the right to self-treat schistosomiasis with praziquantel. In practice, a number of factors may influence a person's decision and ability to do so. Some factors that may influence the decision to self-treat are: the cost of praziquantel, the costs associated with traveling to acquire treatment, and the costs and likelihood associated with receiving an accurate diagnosis of schistosomiasis (Bruun and Aagaard-Hansen 2005, Danso-Appiah et al. 2004). Danso-Appiah et al. (2004) found that most individuals in a survey conducted in Ghana in 2000 did not seek treatment for common symptoms of urogenital schistosomiasis (hematuria, dysuria). Only about 20% of "schistosomiasis signs and symptoms" were considered serious enough by patients to bring the symptoms to the attention of health care workers; "perceived severity of schistosomiasis-related signs and symptoms rarely increased health care seeking, and duration of signs and symptoms and socio-economic status of individuals did neither" (Danso-Appiah et al. 2004). Lack of money to pay for consultation and treatment through the Ghanaian 'cash-and-carry' system, as well as a perception that the signs/symptoms were not sufficiently serious, contributed to the low health care seeking rates (Danso-Appiah et al. 2004).

Integrated Control of Schistosomiasis and other Helminth Infections

In Uganda, populations of schoolchildren (5 to 14 years) were treated for onchocerciasis (ivermectin), schistosomiasis (praziquantel), and intestinal helminths (mebendazole), or for onchocerciasis alone (ivermectin) via three different types of control program (Ndyomugyenyi and Kabatereine 2003). The authors report improved drug (ivermectin) coverage (81.3%) of children involved in an integrated community-directed treatment program (ivermectin and mebendazole on day 1, praziquantel on day 2) over children who were involved in a community-directed treatment (ivermectin only) program (77.2%). Similarly, praziquantel and mebendazole drug coverage were improved (85%) when children were treated through the integrated community-directed treatment program, as compared with children who received praziquantel and mebendazole through a school-based treatment program (79%). The main reason for non-treatment through the integrated community-directed program was insufficient supply of drugs, caused by individuals outside of the target population requesting treatment. Side effects that were mild in nature were reported less frequently by children enrolled in the integrated community-directed program (18%) than by children enrolled in the school-based treatment program (33%). Onchocerciasis, schistosomiasis, and intestinal helminth infections are often co-endemic in poor, rural, tropical areas; thus, Ndyomugyenyi and Kabatereine (2003) show that control efforts can be integrated without adversely affecting drug coverage.

Lack of Equity in Treatment and Prevention Programs

Gordon (2006) analyzed data provided by the Primary School Deworming Project in Kenya and found that in eighteen schools assigned to receive a praziquantel administration program, students who were "…poorer, older, less advanced in school for their age, and from minority households were less likely to be treated for schistosomiasis". Gordon (2006) also conducted a

thorough search of the literature and concluded that schistosomiasis studies consistently fail to consider socioeconomic status or demographics with respect to interventions, despite the World Health Assembly's 2001 call for increased attention to soil-transmitted helminths (STH) and schistosomiasis among the poorest people. Utzinger et al. (2003) have also called for control approaches to specifically target individuals in the poorest communities. Programs selected by health officials to address schistosomiasis may "magnify already existing disparities in endemic societies" (Gordon 2006).

Lengeler et al. (1991) believe that resources for controlling schistosomiasis should be concentrated in high-risk areas. One problem with this approach is due to the focal nature of schistosomiasis; in areas of low endemnicity, there will be heavily infected individuals who will be missed by treatment programs aimed only at people living in high-risk sites, and the DALYs associated with these missed individuals can be substantial. Lengeler et al. (1991) also promote the passive detection of schistosomiasis cases via existing health care systems in low-risk areas. However, it has been shown since publication of Lengeler et al.'s study that passive case detection in some settings is inadequate (Van der Werf et al. 2003a).

Community-Based Praziquantel Distribution

"Within all programmes to strengthen health services, the provision of drugs for the treatment of schistosomiasis and basic laboratory facilities can be considered. In the long term, only effective community-based health services can ensure successful implementation and maintenance of control" (WHO 1993). When schistosomiasis prevalence and morbidity are low, communities can focus on handling clinical cases of disease and on monitoring for introduction or reemergence of infection (WHO 1993). The need for control of schistosomiasis, *as determined*

by a community, should be the deciding factor in terms of establishing infection control priorities (WHO 1993).

Treatment with Praziquantel via the Ghanaian Health Care System

Staff training in endemic countries is necessary for ongoing schistosomiasis control and monitoring of control activities and effectiveness. However, "Training, while essential, will not remedy problems such as high staff turnover or lack of motivation due to poor conditions of service. Low salaries, lack of career development policies, and poor supervision contribute to difficulties in developing and managing disease control programmes in many endemic countries" (WHO 1993).

In Ghana, mass treatment of schoolchildren through The Partnership for Child Development was carried out in the 1990s. Unfortunately, "as in most endemic countries with economic constraints, sustainability of large-scale vertical control programmes has not been achieved" (Danso-Appiah et al. 2004).

The Ghanaian health care system has been evaluated for its ability to correctly handle hypothetical cases of schistosomiasis in local clinics and hospitals (van der Werf et al. 2003a, van der Werf et al. 2004, de Vlas et al. 2004). Van der Werf et al. (2003) interviewed health workers from 70 facilities in 4 different districts. 49 clinics were found to be in schistosomiasis-endemic areas. Symptoms of 4 hypothetical schistosomiasis patients were described to clinic workers with the results that health workers often failed to recognize schistosomiasis symptoms, especially symptoms of intestinal schistosomiasis. Few clinics (11 of 49) in schistosomiasis-endemic areas had praziquantel in stock, and praziquantel would have been prescribed for the 4 hypothetical cases only 37 to 67% of the time without a referral to another clinic. 96% of

interviewed workers knew that hematuria is a symptom of urogenital schistosomiasis, but only 29% of workers prescribed the correct medication (praziquantel) at the correct dose (40mg/kg) (van der Werf et al. 2003a). The probability of actually receiving praziquantel given that one presents at a clinic with blood in the urine is approximately 30 to 32% (van der Werf et al. 2004).

In 2004, de Vlas et al. reported on the probability of receiving praziquantel when passive case detection is used by the national health care system. The authors found that, given bloody urine due to urogenital schistosomiasis, the likelihood of receiving praziquantel is 4.4% in Ghana. This was based on health-seeking behavior of affected individuals, likelihood of prescription of praziquantel by health workers, likelihood of presenting with symptoms given true infection status (estimated to be 50%), and availability of praziquantel at a health facility (de Vlas et al. 2004). De Vlas et al. (2004) concluded that the "main bottleneck" to preventing treatment for people with urogenital schistosomiasis lies with their own health-seeking behavior: few who experience hematuria visit health centers, mainly for financial reasons. But even if this particular bottleneck were addressed, the authors calculate that the other factors listed above would still mean that praziquantel reaches few patients with urogenital schistosomiasis when administered passively via the Ghanaian health care system (de Vlas et al. 2004).

For urogenital schistosomiasis, van der Werf et al. (2003) found that the cost of a diagnostic test, when required by a health worker, averaged US$0.36, and the cost of transportation when a referral was required averaged US$0.72. These costs are substantial when considered alongside the gross national product (GNP) of Ghana; in 1999, GNP per capita was approximately US$353.00 (van der Werf et al. 2003a).

When schistosomiasis is compared with other endemic tropical diseases, such as malaria, affected individuals may be unlikely to receive treatment due to lack of knowledge of the part of

Ghanaian health care providers, the difficulties inherent in obtaining praziquantel, and the costs associated with seeking diagnosis and treatment. The likelihood of treatment may be improved with better staff training at local clinics and a policy normalizing the administration of praziquantel based on reported schistosomiasis symptoms (presumptive treatment), rather than on results of a diagnostic test (van der Werf et al. 2003a).

Bosu et al. (1997) reported on the reasons for low immunization coverage in Ghana. Immunization coverage differs from praziquantel administration in purpose and target audience, but the reasons for low coverage are instructive, nevertheless. Bosu et al. (1997) asked 469 mothers of children under 2 years, 17 immunization service providers, and 10 "heads of health-related sectors" about immunization knowledge, attitudes, and practices. Mothers overwhelmingly believed that it was necessary to bring their children for immunization (93%), but only 26% of mothers were knowledgeable regarding at least 3 immunizable diseases. Community leaders were uninformed about immunizable diseases and the national immunization schedule. Organization of immunization programs at the community level was difficult due to a lack of suitable venue (a school, a nursery, and a cocoa shed were used when available), lack of furniture, lack of privacy, financial difficulties, and transportation difficulties. Community leaders were very poorly informed about the economic situation of mothers with young children and insisted that a moderate fee for immunization services was appropriate, even though it discouraged participation. Most mothers were not deterred from immunizing their children by the possibility of side effects. The 17 service providers claimed to be motivated to work by "the provision of transport, regular supply of vaccines and equipment, salary increase, payment of transport claims, and provision of uniforms" (Bosu et al. 1997).

Korkor Ansah et al. (2009) studied the effect of removing direct payment for medical care in Ghana. The specific outcomes considered were (1) the use of formal health care facilities in Ghana and (2) the prevalence of children with moderate anemia (< 8 g/dl of hemoglobin). The authors found that the prevalence of moderate anemia did not decrease among children who received free health care; however, the use of the formal health care system increased moderately among this same group. The results were unexpected. The authors note that the indirect costs of healthcare have been estimated to be 2 to 3.6 times higher than direct costs in other parts of Ghana and may represent a substantial barrier to improved health outcomes. The use of moderate anemia as the primary health outcome may have been problematic for several reasons: changes in the standard of malaria care; perception of recently-improved treatment effectiveness in communities; and accurate diagnosis of malaria and/or anemia and correct drug prescriptions, among other confounders. The authors concluded that provision of transportation to receive health care and an improvement in the quality of care may be necessary before free health care improves morbidity and mortality among poor populations.

Sarpong et al. (2010) considered health insurance coverage in Ghana. The team found that individuals with low socioeconomic status (SES) tend to live farther from health care facilities and tend to have reduced health insurance coverage than their wealthier counterparts (21% coverage of 'low SES' individuals as compared with 60% of 'high SES' individuals). The authors conclude that "Poverty is a main barrier to access health facilities" (Sarpong et al. 2010), and point out that interventions targeting the entire Ghanaian population preferentially provide services to the wealthy. A separate, targeted effort must be made to reach the poor (Sarpong et al. 2010).

Reinfection with S. haematobium *Following Treatment with Praziquantel*

After treatment with praziquantel, individuals living in endemic areas who continue to contact contaminated surface water are at risk of reinfection with *S. haematobium*. Magnussen et al. (1997) tested populations of Kenyan schoolchildren for 2-years following administration of praziquantel to children testing positive for microhematuria. The populations (total n = approx. 9,000) were distributed throughout 20 schools, but the individual children in each school may have changed over time. Data were aggregated at the level of the school. Such summary data prevents determination of individual reinfection rates. Baseline prevalence of microhematuria ranged from 7% to 63% (mean = 29%) per school. At 1-year post-praziquantel, the prevalence of microhematuria ranged from 11% to 34% (mean = 18%) per school, and at 2-years post-praziquantel, the prevalence ranged from 3% to 23% (mean 11%) per school. The authors do not show data collected directly after praziquantel administration, so it is not possible to distinguish between individuals who cleared the parasite only to become reinfected, individuals who did not clear the parasite, and individuals who were never treated but who were infected at baseline (false-negatives). Children who were infected with *S. haematobium* and enrolled in the schools after the beginning of the study also contributed to a perhaps artificially high estimate of follow-up prevalences of infection. For additional details about each school, see Figure 2 (Magnussen et al. 1997).

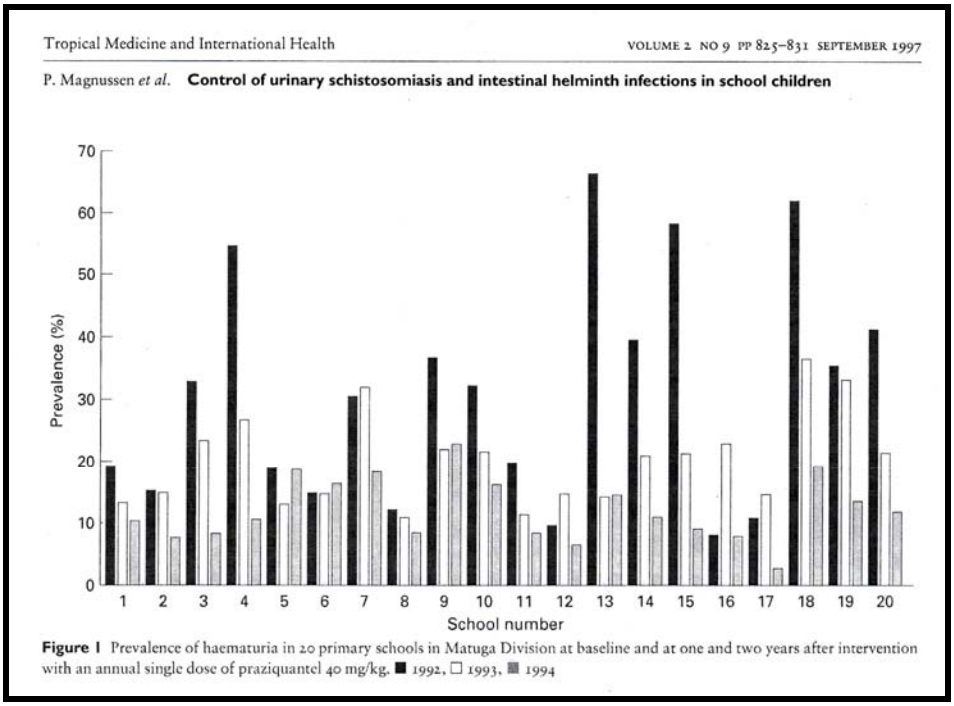


Figure 2: Prevalence of hematuria, a proxy for *S. haematobium* infection, at 20 primary schools in Kenya one year and two years after annual praziquantel administration (40 mg/kg) (taken directly from Magnussen et al. 1997).

In a large population on Pemba Island, Tanzania, Savioli et al. (1989) found that of 24,462 children screened for urogenital schistosomiasis via a simple dipstick test for hematuria, 54% were positive. These positive children were treated with praziquantel (40 mg/kg) and 6 months later, 25,575 children were screened. The authors failed to clarify whether children screened 6 months post-treatment were all screened pre-treatment; the difference in the size of the 2 populations suggests that around 1,000 children screened after treatment were not part of the original study population. Savioli et al. (1989) found that the prevalence of urogenital schistosomiasis among children enrolled in the 52 schools participating in the study decreased

from 54% to 26% after positive children were treated with praziquantel. It is likely that some of the treated children became reinfected after receiving praziquantel, but children were not tested by the authors immediately after treatment to establish the efficacy of praziquantel. It is also likely that some of the children who tested negative during the first screening were false negatives, and thus went untreated. Some of these children may have tested positive during the second screening and may have contributed to a falsely elevated estimate of reinfection.

Urogenital Schistosomiasis: Transmission Control

Morbidity control is currently the strategy of choice to address schistosomiasis, but in some areas where mass treatment has been used successfully, a shift in focus is beginning to occur (WHO 2005). Transmission control ought to be addressed more directly than it has been in the past, in particular because transmission and reinfection rates are not consistently affected by mass administration of praziquantel (King et al. 2006, King 2010b). The Scientific Working Group on Schistosomiasis (WHO 2005) has stated that "transmission is at the root of the problem [of schistosomiasis] and is unaffected by current control activities." Transmission control cannot be achieved through technical solutions alone because transmission is related to social, cultural, behavioral, political, and economic aspects of life in endemic areas (Bruun and Aagaard-Hansen 2005), as well as to the complexity of *S. haematobium*'s life cycle (King 2010b). There is a general consensus that integrated control should be used to halt transmission.

Integrated Control of Schistosomiasis

Around 1983, a consensus was beginning to emerge that schistosomiasis control could best be approached through "integrated programs of control comprising sanitation, provision of safe

water supplies, environmental modification, and public health education" (Tucker 1983). Water and infrastructure improvements that address schistosomiasis transmission include: "bridges over canals, providing a public water supply, and building water facilities for bathing, washing, laundering, and public recreation" (Tucker 1983). More recently, integrated control of schistosomiasis was defined as programs that include drug treatment, water resources management, snail control, and appropriate treatment of human waste (King 2010b). An editorial by Bartram (2008) highlights the connection between water, sanitation, and health; he suggests that health professionals rethink their commitment to improving water, sanitation, and hygiene infrastructure. It is recognized that integrated control efforts designed to reduce reinfection will be "substantially more intensive and expensive" than mass drug treatment strategies (King 2010b). It is also expected that the health benefits will be greater in the long term (King 2010b).

Clennon et al. (2004) suggest focusing control programs spatially and temporally through the use of geographic information systems (GIS) and spatial analyses, and point out that in a community in Kenya, mass treatment with praziquantel and provision of alternative water sources failed to halt schistosomiasis transmission.

Latrines and sanitation may have only limited ability to reduce schistosome transmission if fecal material continues to reach surface water bodies, even if the quantity contaminating the water is relatively small (Macdonald 1965 as cited by Husting 1970).

Schistosomiasis Reinfection Rates

Praziquantel effectively kills adult worms but does not provide protection against reinfection with schistosomes. In endemic areas, if individuals contact contaminated water after treatment, they are likely to be reinfected. Reinfection rates are influenced by geography, behavior, and

ecology, among other factors, and reinfection rates are community-specific. For example, King et al. (2004) estimate that reinfection rates are approximately 15 to 20% per year, and Clennon et al. (2004) suggest that they are between 9% and 21% per year in some parts of Kenya. McGarvey (2005) reports that King has found variations ranging from 3 years to more than 8 years as the median time to reinfection with *S. haematobium* in neighboring Kenyan villages. One reason that mass treatment has failed to stop transmission of schistosomiasis is that some individuals infected with *S. haematobium* will act as "superspreaders" (King 2010b). These individuals are likely to be heavily infected with worms and may engage in risky behaviors, such as urinating in fresh water bodies. Because they are frequently children and young adults who do not attend school (King 2010b), they are difficult to reach via school-based control programs.

Strategies to Address Recreational Contact with Contaminated Water

Despite searching for other studies arguing for construction of public recreation areas, only the article by Tucker (1983) addresses the issue. Senior parasitologists at Noguchi Memorial Institute for Medical Research in Legon, Ghana indicated that recreation-based interventions designed to decrease exposure to cercariae represent an exciting and promising new area of research. The lack of work to date in this area appears to be a conspicuous omission.

Quantitative parasitological techniques should be used to assess novel transmission control strategies (WHO 2005), such as a facility designed to address recreational water contact. When diagnostic techniques are used following an intervention strategy that includes treatment with praziquantel, it can be expected that the egg burden among positive individuals will decrease by nearly 90% post-treatment, making it difficult to find remaining positive cases (WHO 2005).

Human Contact with Water Contaminated by Excreta

Schistosomiasis transmission could be halted if human contact with excreta-contaminated water sources could be prevented, but this goal is unlikely to become a reality for at least two reasons. First, the provision of alternative water supplies to rural communities can be expensive, time-consuming, and technologically difficult or impossible. For example, if excreta contaminated water is contacted when individuals are fishing, it may not be possible to provide an alternative water source that meets community needs. Secondly, if only a few schistosome-infected individuals continue to urinate in community water supplies, the entire community remains at risk of infection (reviewed by King et al. 2006). As described earlier, a single schistosome egg can cause hundreds of cercariae per snail to be produced each day. Each cercaria is potentially infective.

Even in the presence of an uncontaminated water supply and the promotion of sanitation, schistosomiasis may remain endemic if individuals continue to contact contaminated water (Husting 1970). In a comparative study, children living in different communities experienced approximately the same incidence of infection, despite the presence of an improved water supply in some of villages (Tucker 1983). Tucker believes that this phenomenon is because nearly all children were recreating in cercariae-contaminated water bodies, even though clean drinking water was available (1983). Water infrastructure designed to reduce schistosomiasis must be conveniently situated, affordable, dependable, meet daily water needs, be culturally appropriate, and designed to accommodate behavior that normally takes place at surface water bodies in terms of functionality, privacy, convenience, etc. (Husting 1970).

Tchuem Tchuenté et al. (2001) described a study in which, thirteen years after a water pump was installed in a rural village in Cameroon, villagers reported that water contact with cercariae-infested water bodies had dropped dramatically as a result of the new water source. Over this same time period, schistosomiasis infection rates fell to almost 0% from the pre-intervention rates of approximately 19% (*S. intercalatum*) and 12% (*S. haematobium*). These findings seem somewhat remarkable at first, but the authors allow that other factors are likely involved, including repeated treatments with anti-schistosomal drugs (drugs not specified), the natural drying out of snail habitats during some portion of each year, and the absence of a snail host suitable for *S. haematobium*. Regarding the latter point, the snail *Bulinus forskalii* was found locally and is able to host *S. intercalatum*, but not *S. haematobium*. During the study period, *S. haematobium* out-competed *S. intercalatum* following hybridization of the two species, but appeared unable to complete its life cycle locally without an appropriate host (Tchuem Tchuenté et al. 2001).

Characterization of Human Contact with Excreta-Contaminated Water

In areas endemic for schistosomiasis, human contact with water contaminated by excreta can place individuals at risk of contracting the infection. It is necessary to document human behavior with respect to water contact patterns when an intervention designed to reduce water contact is implemented and evaluated. Documenting water contact patterns can be accomplished in a number of ways; the most appropriate strategy (ex. direct observation, self-reporting, a combination of methods, etc.) will likely be community-specific and should be field-tested.

In an area endemic for urogenital schistosomiasis in Nigeria, four major water contact sites were observed once per month from 7am until 5pm. The following information was

collected for each individual seen using the water: "gender, approximate age, activities performed, time spent and parts of body exposed to water" (Oladejo and Ofoezie 2006).

Strategies to Address Human Contact with Excreta-Contaminated Water

Utzinger et al. (2003) discuss the development of sustainable schistosomiasis control strategies, asking whether "previously non-available control approaches and tools [have] been developed that would increase the chances of success". The Bill and Melinda Gates Foundation donated 30 million dollars to the Schistosomiasis Control Initiative (SCI, http://www.sci-ntds.org), partly to fund research into new control strategies (Utzinger et al. 2003). Polderman (1984) suggests the provision of "safe water", or of "barriers" that will prevent human-water contact. It has been suggested that communities restrain children from playing in stagnant waters, or implement a "community crèche" (Mafiana et al. 2003), but no studies have been found in which authors suggest offering a cercariae-free recreation site. Others have suggested that water collected from contaminated sites for domestic use should be allowed to stand for a full day before use because the schistosome cercariae normally do not live more than 24 hours (Stothard and Gabrielli 2007). However, the authors do not suggest that either protective clothing, such as tall boots, be worn while collecting water, or that a platform be constructed to allow people to collect water without entering it.

Snail Control via Molluscicides

Snail control is not usually promoted as an effective means of addressing schistosomiasis transmission. Loker (2005), who is in favor of additional research about mollusk schistosome hosts, points out that "Except in a focal or insular context, it is difficult to imagine how

populations of snails would ever be controlled at a level sufficient to interrupt transmission in a sustainable, cost-effective, environmentally acceptable way."

> "The population dynamics of schistosomes are complex and are not determined by simple proportional rules. Host-related factors, such as human behavior and immunity, as well as ecological factors, such as density of snail populations and infection rates, determine the size and distribution of the parasites population in human communities, which will vary according to the level of endemicity. Consequently, a reduction of transmission by use of molluscicides will not automatically result in a proportional reduction of reinfection rates, particularly in areas with high rates of transmission" (WHO 1993).

Commercially produced molluscicides have been criticized for schistosomiasis control for a number of reasons. First, it is not likely that all host snails will be killed with the chemicals (Tucker 1983, Gryseels et al. 2006, WHO 1993). A study in Egypt cited by Tucker (1983) found that less than 0.3% of snails were infected with schistosome sporocysts, despite the 50% prevalence rate in the nearby human population. Tucker (1983) concluded from this study that a very small number of snails can effectively carry out infection transmission. In 2001, Ndyomugyenyi and Minjas reported that 148 *Bulinus* snails (*B. nastus, B. globosus,* and *B. africanus*) were collected in Tanzania, but none of the snails shed cercariae despite a prevalence of *S. haematobium* infection of 48% (n = 230/483) among local schoolchildren. Clennon et al. (2006) found that less than 3% of *Bulinus* snails shed cercariae but over 30% of individuals living nearby had *S. haematobium* eggs in their urine.

The second problem with molluscicides is that they must be used over a time period that may span years, which is often neither sustainable nor desirable (Tucker 1983, Katsivo et al. 1993b, Gryseels et al. 2006, WHO 1993). Third, molluscicides are often toxic to other aquatic species, such as invertebrates and fish (Tucker 1983, Gryseels et al. 2006), or their eventual environmental impacts are not well known (Katsivo et al. 1993b). Fourth, molluscicides may not be cost-effective given their expense, the efficacy of praziquantel, and the time period over

which they must be applied (Polderman 1984, Katsivo et al. 1993b, Gryseels et al. 2006, WHO 1993). King et al. (2006) call for reconsideration of snail control efforts, citing evidence from the 1950s through 1970s in Japan, China, Iraq, the Philippines, and St. Lucia, but the authors do not address issues of sustainability, toxic chemicals, and alternative options, such as the provision of latrines. In 2010, King again suggested considering snail control in the context of schistosomiasis control programs; however, high cost and need for repeated application of molluscicides, cross-reactivity with other species, negative ecological impacts, and minimal impact on schistosomiasis were mentioned as potential drawbacks (King 2010). Finally, it is difficult to know precisely how to apply molluscicides at ideal locations and times (WHO 1993). Wang et al. (2009) commented on the use of molluscicides and snail habitat modification in China in the past; the authors state that environmental degradation followed these practices.

Health Education about Parasite Life Cycle, Risk Factors, and Treatment Options

Health education is often listed as a critical component of schistosomiasis control, but many studies do not explicitly recognize that in the absence of sanitation and clean water, health education is insufficient. In 2005, the WHO Scientific Working Group on Schistosomiasis stated: "Lack of knowledge, and the poor attitude and practices of most people in schistosomiasis endemic areas, promote the persistence of schistosomiasis transmission despite massive intervention measures" (Kabatereine et al. 2005). The "massive intervention measures" noted by Kabatereine et al. (2005) often amount to little more than mass treatment of populations with praziquantel and rarely – if ever – involve the provision of safe water supplies that meet the needs of target populations. When contact with surface water is necessary for occupational (ex. fishing, car washing, agriculture), hygienic (ex. bathing), or domestic (ex. clothes washing,

dish/utensil washing, house cleaning) functions, health education may serve only to frustrate the poor who cannot afford uncontaminated water; in such cases, "poor attitude" and "lack of knowledge" are not relevant to the discussion.

Ndyomugyenyi and Minjas (2001) found that among Tanzanian schoolchildren (n = 483, aged 5 to 19 years), nearly half of the children had heard of urogenital schistosomiasis, and many (n = 97) "knew that haematuria, dysuria [painful urination], frequent micturition and lower abdominal pain were symptoms" of the disease. As a health concern, the priority of schistosomiasis at the level of the individual may be affected by the presence of other diseases and socioeconomic concerns (Bruun and Aagaard-Hansen 2005). People make conscious decisions about the use of safe versus unsafe water; it is important to understand the factors that constrain these decisions when developing educational or intervention materials to address schistosomiasis (Bruun and Aagaard-Hansen 2005).

Health education serves a number of purposes. First, education can improve local knowledge about the nature of a parasitic infection and the risk of acquiring/transmitting an infection. Second, education can lead to behavior change. Finally, education can help to create an active awareness among people in endemic areas that contributes to sustainability of control programs and ownership of these programs (Bruun and Aagaard-Hansen 2005). "Health education does not work if people have no (attractive) alternatives to unsafe water contact. This observation applies equally to domestic, occupational, religious and recreational activities" (Ekeh et al. 1988 as cited by Bruun and Aagaard-Hansen 2005).

Education may improve knowledge without changing behavior (Kloos 1995 as cited by Bruun and Aagaard-Hansen 2005). It is unlikely that a standard health education program can/should be developed to address schistosomiasis, given that risk factors for infection

transmission vary widely, both geographically and temporally (Kloos 1995 as cited by Bruun and Aagaard-Hansen 2005). A research question that should be addressed is: Do people act on new knowledge about health?, and if so, How? (Bruun and Aagaard-Hansen 2005).

Health education is more likely to be effective if: it is tailored to a specific community; the outcomes are clear and can be quantified; it actively involves community members; and it promotes positive actions, rather than prohibits negative ones (WHO 1993). Health education should be culturally appropriate and should be supplemented by tangible means by which individuals can change their behavior (ex. latrines) (WHO 1993). Community-based health education is usually more effective than health education efforts led by outsiders (WHO 1993).

Community Participation in Transmission Control Programs

Schistosomiasis transmission control should involve behavior change with respect to both water contact and disposal of human excreta (Katsivo et al. 1993b). Katsivo et al. (1993b) conducted a study of community participation in the control of intestinal (*S. mansoni*) schistosomiasis in Kenya. The community cleared canals of vegetation, conducted education campaigns directed at preventing fecal contamination of surface water, dug wells, and build pit latrines in rice fields for use by farmers. Few details about the parasitological impact of the interventions were provided. Katsivo et al. (1993a) presented the results of 2-year post-intervention perception interviews with Kenyan villagers. For unknown reasons, only heads of households were asked to participate in the study. Approximately half of interviewees believed that the community, as opposed to the project team, owned the facilities constructed during the intervention phase. Approximately half of interviewees perceived that management of the facilities was carried out by project field workers instead of by community members. With respect to benefits gained from the project,

most interviewees stated that time and money were saved, health improved, and workload reduced. By the end of the study, knowledge about schistosomiasis had improved dramatically over baseline. The main critiques of the program were: (a) community members should feel a stronger sense of ownership over the project and (b) community members should be trained in operations and maintenance of facilities.

Community participation should mean the inclusion of local stakeholders in the decision-making, planning, implementation, and evaluation processes (Bruun and Aagaard-Hansen 2005). As of 2005, there was a move towards "community-directed" interventions, which contrasts with the objective of simply involving local stakeholders (Bruun and Aagaard-Hansen 2005). In a community-directed model, a community is responsible for identifying a problem and determining an appropriate course of action. Community-directed interventions have outperformed other methods of controlling worm infections (Bruun and Aagaard-Hansen 2005).

It should not be assumed that individual community members or official representatives always act in the best interest of the community (Bruun and Aagaard-Hansen 2005); they are not operating in a social, cultural, or financial vacuum. However, some assumptions have been substantiated by data; for example, "Women generally maintain a higher and more constant level of enthusiasm in surveys and control projects than men" (Feldmeier et al. 1993 as cited by Bruun and Aagaard-Hansen 2005). Finally, it is important to realize that "There is no single universal way to motivate community participation. Success largely depends on factors that differ from village to village, e.g. the authority of the village chief, the internal cohesion of the village community, the general economic situation and economic stratification" (Hielscher and Sommerfeld, 1985 as cited by Bruun and Aagaard-Hansen 2005).

Water, Sanitation, Hygiene and Schistosomiasis Control

Provision of water and sanitation facilities, when combined with laundry, bathing, and recreation facilities are considered costly, but are "the definitive solution to transmission if no zoonotic reservoir exists" (King 2010). It is known that this combination of interventions is likely to impact multiple parasitic infections, but that use of the interventions by target populations is complex and "requires significant behavior changes" (King 2010). In terms of water and sanitation for schistosomiasis control, emphasis should be placed on (a) improving drinking water quality and (b) minimizing human contact with contaminated water (WHO 1993). "To this end, a comprehensive approach considering water supply, excreta disposal, stormwater, domestic drainage, and bathing and laundry facilities is needed" (WHO 1993).

In 1993, the WHO clearly stated that a safe water supply is a necessary, but not a sufficient, component in any schistosomiasis control program (WHO 1993).

> The provision of safe water supplies catering for all domestic needs – not only drinking-water but also washing facilities, cattle watering facilities, and bathing – will reduce not only transmission but also contamination since human contact with natural water bodies is reduced. The provision of latrines in the home is unlikely to affect transmission if people continue to have close contact with natural water elsewhere, whether for domestic activities such as washing or for agricultural or recreational purposes (WHO 1993).

Water supply systems in which communities are responsible for and manage their own systems are preferred over water supply systems in which outsiders are in control, but the funding source for these water supply systems is not clearly stated by the WHO (1993). Immersion-type activities such as clothes washing and swimming contribute to schistosomiasis but may not be adequately altered by the installation of handpumps for drinking water. The design of facilities installed to address these activities should be discussed with the end users (WHO 1993). "Schistosomiasis control programmes are…more sustainable in areas with water supply and

sanitation programmes. The use of schistosomiasis rates as reliable indicators of the impact of water supplies on health is endorsed in all endemic countries" (WHO 1993).

Assessment of Schistosomiasis Control Programs

"Rapid and accurate monitoring of control programmes is essential not only to measure their immediate effectiveness but also to provide information for future advocacy to ensure lasting programme sustainability" (French et al. 2007). Recognizing that assessment must be done and must be accurate is straightforward. However, assessment of schistosomiasis-related morbidity control programs is complicated because after praziquantel is administered, relatively light infections (< 50 eggs/10ml urine) tend to be overrepresented in a population and these can be difficult to diagnose via microscopy and hematuria dipstick tests (Bosompem et al. 2004, Feldmeier and Poggensee 1993). Thus, when interventions such as mass chemotherapy or infrastructure alterations are assessed for schistosomiasis control (WHO 2002), a single screening per individual may be insufficient.

The concept of health disparities is critical to mention here in light of schistosomiasis control programs. Given that morbidity and/or transmission control programs are implemented, "…if health promotion campaigns fail to attend to disparities in the ability to act on available knowledge, they may improve the health of populations with greater access to resources more than the health of populations with reduced access to resources; in other words, they may actually exacerbate, rather than reduce, health disparities" (Schulz and Northridge 2004). Care must be taken at the outset of a control program to ensure that the most vulnerable individuals are not further marginalized and do not experience disproportionately more morbidity than other members of the population.

Indicators of Successful Control Programs

The Scientific Working Group on Schistosomiasis has called for improvements in the type and quantity of data collected by schistosomiasis investigators working on control programs in the field (WHO 2005). The group recommends that researchers develop methods to systematically quantify improvements in health, quality of life, and socioeconomic status, especially as these relate to "anaemia, delayed growth/development, cognitive impairment, and sexual dysfunction…[and] work capacity" (WHO 2005). As part of the Ugandan schistosomiasis (primarily schistosomiasis mansoni) control program, data were collected about improvements in morbidity via questionnaires, ultrasound, and palpation of the liver and spleen (Kabatereine et al. 2005). Growth of schoolchildren in a randomly selected cohort was also assessed longitudinally, as was the affect of treatment on anemia (Kabatereine et al. 2005).

Costs and Benefits Associated with Transmission and Morbidity Control

The WHO (2005) has called for studies to document the cost-effectiveness of morbidity control, as compared with other intervention programs. The cost-effectiveness of various control programs can be compared by calculating the ratio of total resource inputs to program effectiveness (Guyatt and Tanner 1996). Costs should be evaluated comprehensively and in the case of mass treatment with praziquantel, include the financial outflow associated with training and paying staff, shipping goods, wastage/theft of materials, time spent by community members and volunteers, and equipment (diagnostic equipment, vehicles, physical infrastructure). "Capital items" that will be used for more than one year should be priced based on weighted future costs, given an assumed discount rate (usually 3% to 6%) (Guyatt and Tanner 1996).

The ‘effectiveness’ of a schistosomiasis control program is not captured by a straightforward metric, mainly because a control strategy must be defined before defining ways to measure it (Guyatt and Tanner 1996). In the case of a transmission control strategy, the way in which ‘effectiveness’ is defined is an important initial decision; for example, it could be a reduction in egg-counts, a reduction in snail infection rates, a reduction in excreta contamination of water bodies, or any number of other ways in which the schistosome lifecycle is partially or completely interrupted. In the case of a morbidity control strategy, ‘effectiveness’ could be defined by a reduction in bladder pathology as assessed by ultrasound, a reduction in hematuria as assessed by dipstick, an improvement in kidney function, or other measures of improvement in health status. Guyatt and Tanner (1996) recommend characterizing the effectiveness of a program by assessing the long-term reduction in disease. While disease can be assessed by a variety of methods, the use of ultrasound in the field to assess bladder pathology can be valuable, but difficult, for many reasons. While the technique is “safe, rapid and non-invasive”, use of ultrasound requires expensive equipment, highly trained personnel, and good quality control mechanisms in place to ensure reproducibility of results (Vennervald and Dunne 2005). In addition, morbidity is not always visible through ultrasound even when serious morbidity is present (Vennervald and Dunne 2005).

Several authors suggest assessing morbidity from the perspective of an affected person: essentially, the question to be asked is ‘How much ill health is felt by an infected individual?’, in keeping with the definition of “health” as presented by the WHO (King 2005, Vennervald and Dunne 2005).

Schistosomiasis control programs can be cost-effective but still unaffordable at the local point of delivery (Guyatt 2003). Benefits associated with schistosomiasis morbidity control are

often underestimated due to the difficulty of obtaining accurate information about chronic disease (King 2005). Significant morbidity often occurs in subjects aged 20 to 30 years, given schistosome infection a decade earlier (King 2005). The resources required to carry out a morbidity control programs may relate to the cost of:

- Praziquantel
- Praziquantel storage
- Praziquantel transportation
- Administrative assistance
- Costs of diagnosing infection
    - Vehicles
    - Laboratory equipment
    - Personnel
    - Offices
    - Depreciation of capital assets (offices, laboratories, etc.)
- Molluscicide
- Environmental management

Cost estimates for controlling schistosomiasis have been considered from a variety of programs; they range from $0.70 to $3.10 (1993 dollars) per person per year, but usually failed to include "development costs, salaries and the cost of failure" (WHO 1993). Based on the results of pilot control projects, specialized control programs are estimated to cost between $1.50 and $6.53 per person per year. For these programs, the cost per *infected* person treated was higher, at a minimum of $3.00 per person per year (WHO 1993). The use of questionnaires and reagent dipsticks to test for hematuria can reduce the costs associated with identifying communities in

need of praziquantel (WHO 1993); however, there will be a corresponding reduction in morbidity reduction with reduced sensitivity of diagnostic techniques. Similarly, select population groups, such as schoolchildren, can be treated but there will be a reduction in the amount of morbidity prevented at the community level (WHO 1993). The costs of failing to control schistosomiasis include costs associated with health care for affected individuals and due to the lost economic productivity of the affected workers (WHO 1993). The costs associated with deficits in health status, such as those found in DALYs attributable to schistosomiasis, were not discussed by the WHO (1993). The economic analysis for the indirect costs associated with schistosomiasis should be conducted at the level of the household, not the individual wage earner, for rural areas (WHO 1993).

Diagnosis of Urogenital Schistosomiasis

There is no "definitive gold standard reference test for urinary schistosomiasis" (Koukounari et al. 2009). The lack of a gold standard reference test makes it difficult to assess control programs and to accurately estimate infection prevalence (Ratard et al. 1992), especially among populations where the majority of infections are relatively light (Koukounari et al. 2009). Accurate diagnostic tools are essential for characterizing the infection status of individuals and also for determining community-level, country-level, and worldwide prevalence of infection, disease and schistosomiasis-related mortality (van der Werf et al. 2003b). For individuals in a clinical setting, repeatedly screening patients suspected of having schistosomiasis could improve the accuracy of diagnosis and thus, management of the disease.

If the diagnosis of schistosomiasis is to be made by demonstrating eggs in urine, the eggs can be concentrated or filtered from urine in a variety of ways; some of these techniques are

described below (see *Urine Filtration for S. haematobium Eggs*). Typically, 10ml of urine are filtered or concentrated, but the quantity can be increased for improved sensitivity (WHO 1993). In 1993, the WHO recommended that eggs be stained after filtration, but staining is no longer consistently promoted.

In endemic areas, hematuria is a commonly used proxy for infection when demonstration of eggs is not desirable or practical. The rationale behind using hematuria is as follows: the rupturing of schistosome-induced polyps on the walls of the bladder can cause blood to leak from capillaries (see *Pathology of Urogenital schistosomiasis, Chronic Infections*) and microscopic quantities of blood in urine can be detected through the use of simple, inexpensive reagent stick tests (see *Dipstick Test for Hematuria*).

All diagnostic techniques have benefits and drawbacks and the selection of a particular technique or combination of techniques "will therefore reflect a compromise so as to best fulfill the objectives and constraints of a given control programme and should not be regarded as dogmatic" (Feldmeier and Poggensee 1993).

Concentration of S. haematobium *Eggs from Urine*

Although there is no gold standard diagnostic test for urogenital schistosomiasis (Koukounari et al. 2009), schistosomiasis is commonly diagnosed by collecting urine samples in the middle of the day, preferably after exercise, and concentrating eggs from urine via filtration, centrifugation, or sedimentation (Gryseels et al. 2006). Egg counts are usually made following concentration from a fixed amount of urine and counts are typically expressed as eggs/10ml urine (Gryseels et al. 2006). However, it is possible to filter any amount of urine, to record the quantity filtered, and then to express egg counts in the standard fashion (Lengeler et al. 1993). In order to account for

variations in urine sample volumes, Stephenson et al. (1984) suggest multiplying egg concentration (number of eggs/10ml urine) by the total volume of the urine sample submitted, and then dividing this quantity by 100ml. Stephenson et al. (1984) describe this value as "egg count – adjusted", and use it to allow for differences in calculated egg count that result from very large and very small urine samples.

Collection of Urine Samples

A terminal urine sample of at least 10ml in volume should be collected; alternatively, a 24-hour sample can be collected (WHO 2003b). If a urine sample is to be examined under a microscope, it should be fresh and not refrigerated so as to prevent the formation of salts (WHO 2003b). A urine specimen may be preserved by adding 1ml formaldehyde (37%), but this is not necessary. Alternatively, 2ml of household bleach can be used as a preservative (WHO 2003b).

Urine Filtration for S. haematobium *Eggs*

A commonly used method for concentrating *S. haematobium* eggs from urine is filtration of 10ml of urine through a porous membrane (van der Werf et al. 2003b). Filtration is the method of choice when an epidemiological study is performed that necessitates collection of quantitative data (WHO 2003b). The following materials are required for filtration of urine for *S. haematobium* eggs (WHO 2003b):

- Microscope
- Microscope slides
- Coverslips
- Filter holders (13mm or 25mm diameter)
- Membrane filters (12 to 20µm pore size, nylon or polycarbonate or Whatman No. 541 filter paper, or equivalent filter paper)

— Urine sample container
— 10ml plastic syringe
— Lugol's Iodine, 0.5% solution

One membrane filter is placed in a filter holder. The urine sample is gently agitated and 10ml of urine are drawn into the syringe. The syringe is then attached to the holder and the urine expelled through the membrane. The syringe is detached, air is drawn inside, and the air and any remaining drops of urine are expelled through the membrane. The filter holder is then opened and the membrane removed with forceps. The membrane is placed face-down (polycarbonate membrane) or face-up (nylon) on a microscope slide. One drop of Lugol's Iodine can be used to improve the visibility of eggs. 10x or 40x can be used to examine urine for *S. haematobium* ova (WHO 2003b). The reuse of polycarbonate membranes is possible and is described by the WHO (2003b). Specificity of the filtration method approaches 100% (Feldmeier and Poggensee 1993).

In a population of Gambian children aged 2 to 17 years (n = 419), Wilkins et al. (1979) found that reproducibility was good (88.8% correctly matched) with respect to correctly categorizing children as excreting more or less than 200 eggs/10ml urine on two different days. The use of 200 eggs/10ml urine as a cutoff point was apparently arbitrary; the WHO considers 'heavy infection' as 50eggs/10ml urine or more.

Urine Sedimentation and Urine Centrifugation for S. haematobium *Eggs*

To centrifuge urine, 11ml of gently agitated, mid-stream urine is placed in a centrifuge tube. The tube is centrifuged at 2000g for 5 minutes, the supernatant removed, and the pellet re-suspended with distilled water. One drop of the suspension is placed on a glass slide, covered with a coverslip, and examined microscopically at 10x power.

For urine sedimentation, the urine sample is shaken and then allowed to rest for 1 hour in a conical flask. The supernatant is then removed and the pellet transferred to a centrifuge tube. The pellet is centrifuged for a maximum of 2 minutes at 2000g. The pellet is examined is as described for centrifugation. The sedimentation method for examining urine is not as sensitive as filtration through a porous membrane (WHO 2003b).

In 1957, Scott reported on the use of a centrifugation technique to concentrate *S. haematobium* eggs from urine samples in Egypt. 50ml sub-samples of urine "were centrifuged at slow speed long enough to throw down all the eggs" and the sediment was examined for eggs. 28 of 34 individuals who were screened via this method tested positive at least once. Each individual submitted between 4 and 18 urine samples over an unspecified, but "short" time period. Seven of these individuals were found to be negative for *S. haematobium* eggs on at least one occasion. The authors concluded that true positive individuals will occasionally test negative for eggs, that additional data should be collected about this diagnostic limitation, and that "no one experienced with such quantitative methods expects to be able to estimate the intensity of infection of an individual" given egg counts collected from a set of urine samples (Scott 1957).

To diagnose urogenital schistosomiasis in Ghanaian infants, Bosompem et al. (2004) collected 50 to 100ml of urine and centrifuged the samples at 290g. The resulting pellets were then examined and egg counts were reported per 10ml of urine. Asaolu and Ofoezie (1990) fixed 10ml of urine with 40% formaldehyde in a 30ml conical tube and allowed the samples to stand for 4 hours. Sediment in the pointed end of the tube was withdrawn and eggs were counted under a light microscope (40X). Using this technique, the authors recovered over 99% of the eggs; the percent recovery was calculated as eggs recovered via fixation/gravity sedimentation versus total recoverable eggs (eggs via fixation/gravity sedimentation plus eggs via fixation/centrifugation of

remaining supernatant) (Asaolu and Ofoezie 1990). When urine was collected from children younger than 3 years of age in Nigeria, their mothers were given "dark plastic bowls with the instruction to collect any voided urine between the hours…10.00 and 14.00" (Opara et al. 2007). The urine samples were then fixed in 2 drops of 40% formaldehyde; 10ml sub-samples were withdrawn and centrifuged (2000 rpm, 3 min). The pellets were examined for *S. haematobium* eggs. The authors note that using this method, 19.8% of children was egg-positive, but that testing of additional urine samples may have produced a higher prevalence (Opara et al. 2007).

Combination Methods for Testing Urine for S. haematobium *Eggs*

Peters et al. (1976b) describe the use of transparent Nuclepore filters for quantifying the number of *S. haematobium* eggs in saline solution in the laboratory. The advantage over older techniques is that egg staining is unnecessary (Peters et al. 1976b). In the laboratory, known concentrations (10, 100, 1000, and 10,000 eggs/100ml 0.9% saline solution) of *S. mansoni* eggs were passed through Nuclepore filters (13mm, 8µm). The same process was repeated for *S. haematobium* and *S. japonicum* eggs, but tests were conducted only with eggs at a concentration of 1000 eggs/100ml 0.9% saline solution. Multiple sub-samples (n = 5 to 11) of various volumes (0.1, 1.0, and 10ml) of egg/saline solution were injected through the Nuclepore filters. Filters were placed face-down on glass slides and examined microscopically (40X). 23% of samples (n = 52) containing an expected 1 egg were negative. All samples containing an expected 10 or 100 eggs were egg positive (Peters et al. 1976b).

The laboratory method was adapted for field use by Peters et al. (1976a). Urine samples were collected from 510 Kenyan schoolchildren aged 6 to 18 years. Sub-samples of urine (1ml, 5ml, and 10ml aliquots) were withdrawn from collection containers via a plastic syringe from

which the needle had been removed. Urine was forced through Nuclepore membranes (13mm, 8μm), and then air was pumped across the filter (Peters et al. 1976a). Eggs were counted as described in the laboratory method (Peters et al. 1976b). Plastic syringes were washed in a detergent solution, rinsed twice, and reused. Cross-contamination caused by eggs remaining on syringes did not occur (Peters et al. 1976a). Several sub-samples of urine were tested for each child: 10ml and 1ml aliquots, 5ml and 1ml aliquots, and 5ml and 5ml aliquots were sampled in pairs. When eggs were present, and the egg concentration in the 10ml and 5ml sub-samples was less than 1egg/ml urine, all 1ml sub-samples of urine were egg-negative. All 1ml sub-samples were egg-positive when the concentration of eggs in the 5ml and 10ml sub-samples was greater than 1egg/10ml urine. Egg counts from "different volumes [of urine] were proportional," indicating that volumes of urine larger than 10ml can likely be filtered without loss of comparability to methods in which 10ml of urine are filtered. The mean egg counts established by the 5ml aliquots taken from the same urine sample were not significantly different from each other, indicating that the distribution of eggs in the urine samples was relatively homogeneous (Peters et al. 1976a).

Stephenson et al. (1984) conducted testing on a population of "previously unscreened" Kenyan schoolchildren (n = 359) aged 6 to 16 years in 1981 and 1982. The urine test was performed as described by Peters et al. (1976a), with the exception that one drop of 0.5% trypan blue dye in saline solution was added to each filter directly before eggs were counted (Stephenson et al. 1984). Feldmeier et al. (1979) also used a trypan blue staining method; when individuals were suspected to be excreting few eggs, the entire volume of a urine sample was filtered instead of the typical filtration method of a 10ml sub-sample. Feldmeier et al. (1982)

screened children on four consecutive days to establish presence/absence of infection, but did not report the variation in infection status from day-to-day.

In field tests conducted in Ghana, Bosompem et al. (1998) collected urine samples from schoolchildren aged 6 to 20 years (n = 155) on 9 days over the course of 2 weeks. 10ml of each sample was filtered through a Nucleopore membrane (25mm, 12μm). The remaining urine was centrifuged (400g) and the pellet was examined microscopically (Bosompem et al. 1998).

10ml of urine from samples collected in South Africa by Saathoff et al. (2004) were filtered on the same day as collection as described by Peters and Kazura (1987) and then stained with 50% Lugol's iodine saline solution. Egg counts were made by 3 different individuals, and 5% of the slides were examined twice, presumably for quantification of inter-observer variability (Saathoff et al. 2004). While staining no longer appears to be a common practice, it was used in the late 1970s, partly because filter paper was not transparent (Pugh 1978). Clear Nucleopore filters are now widely used, and so the expense and time associated with sample staining render this step unnecessary to carry out in the field.

Schutte et al. (1994) compared the use of a Visser filter with the results of urine filtration through polycarbonate membranes. Visser filters are not widely in use, possibly due to the amount of time required to prepare and process a sample, but they are capable of handling entire urine samples, as opposed to 10ml sub-samples. Schutte et al. (1994) found that Visser filtration of 190ml of a 250ml urine sample characterized 14.7% of samples as true-positives when the same samples tested egg-negative via membrane filtration of a 10ml sub-sample. Samples that were false negatives by membrane filtration and true positives by Visser filtration were typically samples with small numbers of eggs (Schutte et al. 1994).

Microscopy for S. haematobium *Egg Counts*

There is a practical upper limit to the number of eggs per slide that can be reasonably counted. Feldmeier and Poggensee (1993) suggest that the range of 50 to 100 eggs represents the upper limit of the number of eggs that can be counted on a single microscope slide. An egg is viable if the miracidium or flame cells are moving; for further details, see WHO (2003b).

Urine Dipstick Test for Hematuria

The movement of schistosome eggs through tissue en route to excretion from the body can induce immune-system mediated inflammation, thickening of and polyp formation on bladder walls, and tears in vascular tissue. These processes may cause hematuria, which is a common symptom of urogenital schistosomiasis (Coon 2005). Microscopic quantities of blood are often found in the terminal urine (called microhematuria) stream from infected individuals (Gryseels et al. 2006), but in some cases, the entire urine sample is visibly red, brown, or cloudy (called macrohematuria, or gross hematuria). Savioli et al. (1990) found that the specificity of macrohematuria for urogenital schistosomiasis approached 100%. Inexpensive dipsticks are commercially available to test for hematuria; presumptive diagnosis of schistosomiasis can be made on the basis of an individual reporting hematuria or by detecting hematuria via reagent dipstick (WHO 1993).

Many, but not all, people infected with *S. haematobium* will present at least occasionally with hematuria, but the presence of blood in the urine is subject to temporal variation, and hematuria is not schistosomiasis-specific. For example, false-positive cases of urogenital schistosomiasis may result if a tested individual has a genito-urinary tract infection (Bosompem et al. 2004). There is no linear relationship between infection intensity as determined by egg

count and degree of hematuria (Feldmeier and Poggensee 1993). Feldmeier and Poggensee (1993) state that it is critical to calibrate the dipstick test for each schistosomiasis-endemic setting.

The dipstick test for hematuria is typically carried out between 1000 and 1400 hours because this is the known period of time for peak egg excretion; however, it important to note that at least one study (Doehring et al. 1985) has found that the peak of erythrocyte excretion occurs much later in the day, at approximately 1800 hours. These results were based on a sample size of 5 boys aged 7 to 9 years who were followed extensively over the course of 5 days.

Sensitivity and Specificity of Urine Dipstick Test

The sensitivity and specificity of a diagnostic test, such as the dipstick test for microhematuria, can be calculated and used to describe test performance, but it is important to note that sensitivity specificity for any indirect test for schistosomiasis are not constant. They are influenced by factors such as environmental conditions, demographics, and sex, among others (Feldmeier and Poggensee 1993). Sensitivity and specificity are defined as follows:

$$Sensitivity = \frac{(\text{Number of True Positives})}{(\text{Number of True Positives} + \text{Number of False Negatives})}$$

$$Specificity = \frac{(\text{Number of True Negatives})}{(\text{Number of True Negatives} + \text{Number of False Positives})}$$

The use of a dipstick test for microhematuria is appropriate after the dipstick has been calibrated based on urine tests for *S. haematobium* eggs (Mott et al. 1985). Where egg counts are expected to be relatively low, more than one egg count per person may be necessary for correct interpretation of dipstick results. For example, a dipstick test was considered to have produced a false-positive ratio of 50% following filtration of a single 10ml sub-sample of urine (Eltoum et al. 1992). However, some of the blood-positive, egg-negative individuals in the study responded well to praziquantel, indicating that these may have been schistosomiasis cases (Eltoum et al. 1992). In another study, a dipstick test (1+ lower test limit) was considered a false positive in the absence of eggs (via a single test of centrifugation, sedimentation, or filtration of 5 to 20ml of urine), a definition that was used in a meta-analysis to compare 4 diagnostic methods for urogenital schistosomiasis (Van der Werf and de Vlas 2004). In a series of 9 studies conducted at 9 schools in Tanzania between 1984 and 1988, the sensitivity of a single dipstick test (compared with a single urine filtration test, total volume of urine filtered) ranged from 33.3 to 88.5% when a 1+ blood reading lower limit was used (Lengeler et al. 1993). Specificity ranged from 67.6 to 98.9% with an imposed 1+ blood reading lower limit (Lengeler et al. 1993). Sensitivity, specificity, and positive and negative predictive values were calculated for microhematuria by Ndyomugyenyi and Minjas (2001) for a Tanzanian cohort (schoolchildren aged 5 to 19 years): respectively, they were found to be 84%, 71%, 77%, and 84%, based on comparison with egg counts from a single urine sample per person. The authors note that 58 children tested positive for microhematuria and negative for eggs. Of these 58 children, 15 tested positive for eggs 8 weeks later, indicating that they may have been true positives during the initial survey (Ndyomugyenyi and Minjas 2001). French et al. (2007) also tested the sensitivity, specificity and

positive and negative predictive values of the dipstick test. However, the calculations were again made based on the egg readings from filtration of one 10ml urine sample per person.

The sensitivity of the dipstick test for blood has been shown to improve as mean egg intensity increases (Feldmeier et al. 1982, Lengeler at al. 1993, Ndyomugyenyi and Minjas 2001, Stephenson et al. 1984). In agreement with these findings, Savioli et al. (1990) found that two urine filtration tests per person were needed before the filtration-estimated prevalence of urogenital schistosomiasis was close to the prevalence estimated via a single dipstick test for microhematuria. Savioli et al. (1990) concluded that, in the Tanzanian community studied, a single dipstick test is more effective at finding heavily infected individuals than is a single filtration test. Stephenson et al. (1984) tested a sub-population of Kenyan schoolchildren (n = 244) twice, once before and once after administration of metrifonate, and found that dipstick test (trace level of blood = positive) sensitivity decreased from 88% pre-treatment to 64% post-treatment, and specificity decreased from 97% to 87%. These results strongly suggest that the sensitivity and specificity of the dipstick test should be evaluated in each endemic setting prior to interpreting results, as individuals with light *S. haematobium* infections may be missed by dipstick and filtration techniques, given single-day testing.

Savioli found that 20% of individuals who presented with eggs at least once never presented with microhematuria. In general, these individuals were lightly-infected, which was defined by the authors as having consistently low egg counts (< 50 eggs/10ml urine) and as testing negative on some of the test days. Stephenson et al. (1984) report similar findings: 24% of children with 1 to 29 eggs/10ml urine were hematuria-negative. Van der Werf and de Vlas (2004) report that when *S. haematobium* eggs are found in 50% of samples from a population,

only 38% of individuals will present with microhematuria. It is not clear whether the 38% who present with microhematuria also present with eggs, or whether some are egg-negative.

Feldmeier et al. (1982) used three dipstick tests per person for proteinuria, hematuria, and leukocyturia to diagnose schistosomiasis. The combination of these three tests, termed the "reagent strip index", was found to correlate with the intensity of infection as assessed by four consecutive screenings for *S. haematobium* eggs. After treatment of study subjects with Metrifonate, the correlation was diminished. Even before treatment, Feldmeier et al. (1982) note that the number of false negatives (lack of sensitivity) with the reagent strip test for hematuria alone was "unacceptably high."

Agreement between Urine Dipstick Test and Egg Filtration Test

Lengeler et al. (1993) compared the prevalence estimates made via a single urine filtration test with the estimates made via a single urine dipstick test and found that the two methods were usually comparable. 1,429 children were screened in 9 different studies between 1984 and 1988 in Tanzania. Up to 60ml of urine from each child was filtered once, and a single dipstick test was conducted per child. Linear regression was used to evaluate the prevalence estimates by these two methods. Correlation was significant ($r = 0.90$, $p < 0.001$) when blood at the 1+ level was used, but the slope of the regression equation was low ($Y = 0.68X + 4.85$) and no evaluation was shown that validated the significance of the slope or intercept in the final model. Correlation improved ($r = 0.96$, $p < 0.0001$) when blood at the 2+ level was used ($Y = 1.02X + 4.64$), but again, no evaluation of slope/intercept significance was shown.

Variation in Results of Urine Dipstick Test by Brand

Reagent dipstick tests may produce results that differ by brand. Lengeler et al. (1993) found that Combur 9^{TM} were 1.2 times more sensitive for hematuria than were Hemastix^{TM}; this difference was significant ($\chi^2 = 18.1$, $p < 0.001$) at the 1+ blood detection limit. The correlation between reagent stick test value (0, 1+, 2+, or 3+) and mean egg intensity has been shown to vary by location (Tanner et al. 1983 and Mott et al. 1985, as cited in Lengeler et al. 1993).

Use of Dipstick Test by Non-medical Personnel

Dipsticks can be used effectively by non-health personnel, such as teachers working in schools in endemic areas (Lengeler et al. 1991). Lengeler et al. (1991) found that dipstick test results were quite accurate when performed by minimally trained school teachers in 73 schools in Tanzania. A cross-check confirmed that teachers' results were reproducible (Pearson's correlation coefficient = 0.98, $p < 0.0001$). However, the accuracy of the dipstick test was not confirmed by the demonstration of *S. haematobium* eggs and so the sensitivity, specificity, and predictive values of the dipstick test were not calculated.

Limitations of Urine Filtration and Urine Dipstick Tests

The WHO (2002, 2005) has called for improved diagnostic techniques for detecting schistosomiasis. There is substantial day-to-day variation and intra-sample variation in egg counts (Savioli et al. 1990, Bosompem et al. 1998). Feldmeier and Poggensee (1993) reviewed work stating that the spread of *S. haematobium* eggs in urine probably follows a Poisson distribution; the probability of finding an egg in a urine sample can be estimated by the following equation, where s = 'sample size' and d = 'density of ova in sample': $p = 1 - e^{-sd}$.

The WHO (2000) states that one of the major challenges facing schistosomiasis control today is the difficultly of accurately diagnosing low-intensity infections in areas where control programs have been used. In low-transmission areas, the WHO (2000) reports that the sensitivity of urine testing can be improved "by repeated sampling and/or sampling of a larger volume of…urine", but that such methods are not usually practical with large populations. It is one of the objectives of this thesis to show that these methods are practical to implement in intervention studies, and they should be used to increase accuracy.

The WHO (2005) has called for interdisciplinary work on the cost-effectiveness of various combined approaches to detecting schistosomiasis cases. The idea is to minimize costs and maximize the number of individuals correctly identified and treated through "inter and trans-disciplinary (bio-science, mathematics, epidemiology, programme management) research".

The prevalence of urogenital schistosomiasis in a population may be higher than is reported via the evaluation of a single urine sample (Bosompem et al. 1998, Bosompem et al. 2004, Mafiana et al. 2003, Ndyomugyenyi and Minjas 2001, Opara et al. 2007, Saathoff et al. 2004). This may be particularly true among lightly-infected individuals or among individuals excreting few eggs. It has been known for some time that the number of eggs excreted by an individual infected with *S. haematobium* is highly variable, and the irregularity of egg excretion does not appear to show a predictable pattern (McCullough and Bradley 1973). McCullough and Bradley (1973) found no predictable pattern of egg excretion at the individual level, but children who excreted a high number of eggs one year were likely to excrete a high number of eggs in two subsequent years, and vice-versa for those excreting few eggs; these follow-up studies were carried out in the absence of treatment for affected children. Children with few eggs in their

urine were particularly likely to have highly variable egg counts over the course of several urine screenings in a short period of time (McCullough and Bradley 1973).

Some studies have found that females tend to have lighter infections than males, and thus, these tests may underestimate infection prevalence among women (Bosompem et al. 1998, Ndyomugyenyi and Minjas 2001). In writing for the Report of the Scientific Working Group on Schistosomiasis, McGarvey (2005) noted that schistosomiasis "transmission remains active in many areas of the world with a likely increase in the proportion of individuals infected at low intensity levels." This likely increase in individuals with relatively light infections – whether as a result of treatment with praziquantel or for other reasons – should be cause for concern among those carrying out fieldwork to estimate true disease prevalence, given the present state of poorly-characterized limitations of common field testing methods.

Hatz et al. (1998) tested urine samples from Tanzanian schoolchildren (n = 533) on 5 consecutive days for eggs and blood, but reported only overall prevalence and not the number of days that a child was positive by either or both of the tests. Testing for *S. haematobium* eggs was accomplished by filtering 10ml of urine through a Nucleopore membrane (12μm pore size) and a semi-quantitative reagent strip was used to test for hematuria (Hatz et al. 1998).

One study was found that quantifies the minimum "number of daily egg counts in urine necessary to establish intensity of infection" (Warren et al. 1978). However, infection 'intensity', as determined by egg count, is not necessarily an accurate estimate of worm burden (Hall 1982). Thus, the 1978 study by Warren et al. can be considered a study of the reproducibility of egg counts, but not necessarily a study of "infection intensity".

In the study, 121 Kenyan children aged 7 to 15 years were tested for *S. haematobium* eggs via filtration through Nuclepore filters (13mm diameter, 8μm pore size) on 10 school days

over the course of two weeks. Different size aliquots of urine were tested (10ml, 5ml, 1ml) for each child based on the results of triplicate testing (10ml, 5ml, 1ml sub-sample volumes) on days 1 and 2 of the study. Children with few (not defined) or no eggs on the first two days of testing were tested via 10ml sub-samples for days 3 through 10; children with moderate egg counts (not defined) were tested with 5ml sub-samples, and children with high egg counts (not defined) were tested with 1ml sub-samples. All children were tested in duplicate on days 9 and 10 of the study. 72% of children submitted all 10 requested urine samples, but day 1 data were excluded from analysis for all children (Warren et al. 1978).

Overall infection prevalence in the population studied by Warren et al. (1978) was 81%, based on 9 days of sampling. 53% of the population was heavily infected (≥ 50 eggs/10ml urine) based on the overall mean egg count following 9 days of sampling. There were 34 lightly infected children (mean egg count < 50 eggs/ml, tested positive at least once) in the study, and 23 children who were egg-negative on all 9 test days. Of the 34 lightly infected children, 26 of them (76%) were egg-negative at least once (range = 1 to 8 days). 21 of the 34 children (62%) tested egg-negative on 5 to 8 days, out of the 9 possible test days. Of 306 urine samples collected from children who were egg-positive at least once and had mean egg counts less than 50 eggs/10ml urine, 90 (29%) were egg-negative. The authors concluded: "a random urine specimen is likely to have eggs present unless the subject is very lightly infected", where "very lightly infected" is defined as having a mean of less than 50 eggs/10ml urine (Warren et al. 1978), but based on the data described above, this statement may be somewhat misleading. It has been argued that "light" infections (by WHO definition, < 50 eggs/10ml urine) should be taken seriously, given that a host's immune response to eggs determines pathology, and not the number of worm pairs or eggs present (Hall 1982).

A study by Bosompem et al. (1998) evaluated the likelihood of correctly identifying true positive and true negative individuals via the urine filtration test for eggs, but the novel test used by the authors is not commercially available and has not been independently verified. The Scientific Working Group on Schistosomiasis notes that a "paradoxical outcome of the reduction in morbidity is the dearth of improvements in available control tools…even if research has generated various novel serological assays of potential application, the fall in intensity of the disease has not been met with more sensitive diagnostic capabilities in the field" (WHO 2005). Another WHO (2002) document also states that the lack of improved sensitivity of diagnostic tools is a major problem in light of diminishing morbidity due to mass praziquantel administration. It is one of the objectives of this thesis to contribute to the literature describing the sensitivities and specificities of two test methods (urine filtration and dipstick test for hematuria) commonly used in the field. An improvement in the sensitivity of field testing methods – or at least a quantification of the limitations of field methods – is important not just from the perspective of an individual who may require treatment (King 2010). Accurate characterization of disease prevalence and intensity in a population is essential for the testing of new drugs, infrastructure alterations, and control programs (WHO 2005).

Utzinger et al. (2001) studied the limitations of stool examination for *S. mansoni* eggs, given that this is a common method of diagnosing intestinal schistosomiasis. Intestinal schistosomiasis is most commonly caused by *S. mansoni* and *S. japonicum*. The authors found that examination of a single stool sample caused significant underestimation of infection prevalence when compared with the results of examining five stool samples. Examining the same stool sample more than once, in different locations, also improved the sensitivity of the diagnostic test. The authors concluded that day-to-day variation in egg output by adult *S.*

mansoni worms is substantial and becomes even more important following praziquantel administration.

The absence of eggs in a urine sample does not indicate that an individual is uninfected with *S. haematobium* (Scott 1957). It has been shown that infection prevalence is more likely to be underestimated in treated communities than in populations that have never received praziquantel (Bosompem et al. 2004, Saathoff et al. 2004, Stephenson et al. 1984, WHO 2000, WHO 2005). In such cases, the overall prevalence of schistosomiasis will often decrease, but low-intensity infections will be overrepresented. The sensitivity and specificity of the filtration test vary according to the experience and skill of the laboratory technician carrying out the test (Brinkman et al. 1988). Lightly-infected individuals, or individuals excreting few eggs, are likely to be missed by egg concentration techniques (Bosompem et al. 1998, Coon 2005, Feldmeier et al. 1982, Feldmeier and Poggensee 1993, Gryseels et al. 2006, Stephenson et al. 1984).

Monoclonal Antibody-based Dipstick-ELISA (Enzyme-linked Immunosorbent Assay)

An ELISA-based dipstick assay was developed to test for urogenital schistosomiasis (Bosompem et al. 1998). Field trials of this dipstick were conducted on urine collected from schoolchildren aged 6 to 20 years (n = 155) in Ghana. Results of the dipstick-ELISA were compared with results from microscopy following 9 days of testing over a 2-week period. The specificity of the dipstick was found to be 92.2% (119/129). 8 of the 10 individuals who were positive by the dipstick-ELISA and negative by microscopy were females with suspected light infections. Over the course of 9 days, sensitivity ranged from approximately 70% to 100% among 36 known-positive study participants.

Questionnaire for Macrohematuria

Van der Werf et al. (2003b) examined other studies that used questionnaire data with a recall period of 2 weeks. The objective was to determine the prevalence of hematuria and dysuria among *S. haematobium* infected people. Lengeler et al. (1991) tested a two-step method in which members of the district education department screened children for schistosomiasis via a questionnaire and via a dipstick test for microhematuria. The questionnaire method was sensitive, specific, and had high positive and negative predictive values (all scores > 81.8%) in populations with moderate to high prevalences of schistosomiasis. The results of the dipstick test for hematuria were used as the standard against which the questionnaire was evaluated (Lengeler et al. 1991). The effectiveness of a questionnaire in a low-transmission setting area is unclear.

Guyatt et al. (1999) evaluated the use of school-based questionnaires administered by teachers in terms of diagnostic accuracy. The comparison standard was parasitological analysis of a single urine sample per child. The urine sample was tested via a sedimentation/centrifugation method (details not provided). Based on the entire study area (52 schools, both sexes, 3,928 children), the authors concluded that the mean sensitivity of the questionnaire method was 51.5% (range 18.8 – 82.6%) and the mean specificity of the questionnaire was 79.2% (range 53.3 – 96.7%). Overall mean prevalence of urogenital schistosomiasis in the study area was 58.1% (range 15.7 – 85.3%). The mean estimate of sensitivity was lower (33.1%) when only schools with children self-reporting less than 30% hematuria were considered. In these schools, the mean sensitivity estimate was higher (87.0%). In general, sensitivity of self-reported blood in urine was much poorer for girls than for boys. The authors concluded that "if reported blood in urine is used in low-prevalence schools to select

individuals for treatment, then only a quarter of infected girls will be identified compared to 41% of infected boys" (Guyatt et al. 1999).

Rationale for Improved Understanding of Accuracy of Diagnostic Tests for S. haematobium

In addition to new techniques, the WHO (2005) states that the "the best scientific contributions and progress over the last decades have resulted not in the development of new methods but in better understanding of the advantages, limitations and utility of available methods." It is critical to quantify the underestimation of infections for a number of reasons. First, the WHO (2005) has proposed a change in the focus of schistosomiasis control programs from a 'morbidity control' strategy to a 'transmission control' strategy (WHO 2005). An improved understanding of the limitations of field diagnostic techniques would allow accurate assessment of infection prevalence (Scott 1957), especially following transmission control programs. Accurate prevalence estimates will enable project managers to calculate the efficacy and cost-effectiveness of various transmission control strategies. The same rationale can be applied to the testing of vaccine candidates and treatment regimens and to characterization of the true DALYs associated with schistosomiasis (King 2010, WHO 2005).

CHAPTER 2: HYPOTHESIS AND OBJECTIVES

This study was conducted in Adasawase, a community in rural Ghana. Adasawase is located in the Eastern Region and has a population of approximately 2,000 people. There are approximately 500 school-aged (6 to 15 years) children in the community. Community members mainly practice subsistence farming or small-scale commercial agriculture. Water for domestic use is available at approximately seven hand-pumped boreholes at various locations in the community. One surface water body, a small, slow-moving stream called Tini River flows near the town; it is a popular recreation site for school-aged children. Schistosomiasis was identified as a potentially prevalent infection by the chief of the community, Osabarima Kwame Tia II. In December 2007, Osabarima Kwame Tia II invited our study team to the community to screen children and to collaborate with the community to find solutions to the problem of *S. haematobium* infection.

Hypothesis

Children in Adasawase, Ghana who play and wash at a water recreation area will have lower burdens of urogenital schistosomiasis by egg count as compared with children who predominantly play and wash in local rivers and streams. It is expected that disease burden over time will be proportional to a child's use of excreta-contaminated river water. It is reasonable to believe that a water recreation area will support the decreased use of river water for recreation and bathing. If schistosomiasis prevalence is shown to decrease in the presence of the water recreation area, the recreation area may represent a new tool for the primary prevention of urogenital schistosomiasis among young, rural populations.

Objectives

The following objectives have been accomplished in the course of testing the thesis hypothesis:

1. In June 2008, urine from schoolchildren was tested on 3 occasions in two weeks via (a) dipstick for microhematuria and (b) membrane filtration for eggs. The goal was to accurately determine the pre-intervention prevalence of *S. haematobium* infection among schoolchildren in Adasawase. A comparison of prevalence as assessed by single-day and multi-day testing was made. All children in the study area were offered praziquantel by health workers from Ghana Health Services according to WHO standards.
2. Starting in January 2007, design for a water recreation area commenced. The design represents a collaborative effort among schoolchildren, college graduate and undergraduate students, engineering and health professionals, and local community members. Individuals involved in design and construction are nationals of Ghana and the United States. The goal was to design a structure that (a) meets community needs, (b) is economically appropriate, and (c) is attractive to users. Construction of the recreation area was completed through a cooperative effort involving the research team and community members in June, July and August of 2008 and January, June and July of 2009.
3. In June 2009 and June 2010, schoolchildren were tested up to 4 times for *S. haematobium* infection and incidence rates in Adasawase in the two years were compared. Children in the study area were treated with praziquantel by health workers from Ghana Health Services according to WHO standards.

4. From July 2009 through November 2009, children in the study area were observed at the Tini River, the only potential exposure point in the community, to characterize their behavior with respect to surface water in Adasawase, Ghana.
5. Logistic regression models were developed to characterize risk factors for *S. haematobium* infection in the presence and absence of the water recreation area.

CHAPTER 3: DIAGNOSTIC ACCURACY OF URINE FILTRATION AND DIPSTICK TESTS FOR SCHISTOSOMA HAEMATOBIUM INFECTION IN A LIGHTLY-INFECTED POPULATION OF GHANAIAN SCHOOLCHILDREN

Abstract

Two screening methods, reagent dipsticks for hematuria and urine filtration for *Schistosoma haematobium* eggs, were evaluated for their sensitivity and specificity in diagnosing infection with *S. haematobium* in lightly-infected Ghanaian children. Schoolchildren aged 8 to 18 years (n = 255) provided urine samples on three occasions. Overall, 36.4% of girls and 50.7% of boys presented with eggs at least once; 3.3% of girls and 7.5% of boys presented with both eggs and hematuria three times. Many children presented with eggs but without hematuria, or with hematuria but without eggs. When each child was screened three times, the sensitivity of each test method improved by at least 22.9% as compared with single screening, but previously unidentified infections were detected at the third screening, indicating that even three screenings is insufficient. Nearly half of lightly-infected children (< 50 eggs/10ml urine, by maximum egg count) were egg-positive during only one of three screenings. Thus, data presented here indicate that when individuals are screened repeatedly, infection status can be assessed more accurately, control programs can be properly evaluated, and population estimates of *S. haematobium* infection may be made with increased confidence, as compared with single screening.

Key Words

Urogenital Schistosomiasis, Urinary Schistosomiasis, Diagnostic Accuracy, Urine Filtration, Hematuria

Introduction

Urogenital schistosomiasis, caused by *Schistosoma haematobium*, is commonly diagnosed by demonstrating parasite eggs in urine. A variety of diagnostic methods exist, but results are typically expressed as eggs/10ml urine (Gryseels et al., 2006). There is no standard reference test for urogenital schistosomiasis (Koukounari et al., 2009). A better understanding of the limitations of two commonly-used diagnostic tests, urine filtration for *S. haematobium* eggs and a dipstick test for hematuria, would improve reporting accuracy for control programs (WHO, 2005), estimates of vaccine efficacy (WHO, 2005), clinical diagnosis of schistosomiasis, calibration of tests for specific field settings (Feldmeier and Poggensee 1993, Mott et al., 1985), and accuracy of worldwide estimates of *S. haematobium* infection (King 2010, WHO2005).

Infection prevalence based on egg enumeration tests is more likely to be underestimated in communities recently treated with praziquantel than in communities that have never received the drug (Stephenson et al., 1984; WHO 2000; Bosompem et al., 2004; Saathof et al., 2004; WHO 2005). In recently-treated populations, overall infection prevalence may decrease but 'light' infections (WHO definition: < 50 eggs/10ml urine) may be overrepresented. This is a major challenge in schistosomiasis control (WHO 2000; WHO 2002); 'light' infections should be taken seriously because a host's immune response to eggs, and not the number of worm pairs or eggs present, determines pathology (van der Werf et al., 2003). Moreover, there is no linear relationship between infection intensity as determined by egg count and degree of hematuria, which is a proxy for morbidity (Feldmeier and Poggensee 1993).

Urine filtration through a membrane is a common egg concentration technique, but the prevalence of *S. haematobium* infection is higher than what is reported following a single screening (Feldmeier et al. 1979, Bosompem et al., 1998; Ndyomugyenyi and Minjas 2001;

Mafiana et al., 2003; Bosompem et al., 2004; Saathof et al., 2004; Opara et al., 2007), particularly among individuals excreting few eggs (McCullough and Bradley 1973, Stephenson et al., 1984; Bosompem et al., 1998; Coon 2005; Gryseels et al., 2006, Warren et al. 1978). The sensitivity of urine testing can be improved "by repeated sampling and/or sampling of a larger volume of…urine", but such methods are considered "not practical" (WHO 2000). In testing a monoclonal antibody assay, Bosompem et al. (1998) showed that up to nine repeated examinations of urine via filtration and centrifugation were necessary before any given infected individual was correctly identified. However, studies assessing the sensitivity and specificity of urine filtration have not been found. With the abundance of light infections that are seen today in the wake of mass praziquantel distribution campaigns, it is essential that the diagnostic accuracy of these tests be accurately characterized (King 2010, McGarvey 2005, WHO 2002).

Hematuria, a common symptom of urogenital schistosomiasis, occurs when *S. haematobium* eggs induce inflammation and blood vessel rupture (Coon 2005). Although reagent dipsticks are available to test for hematuria, which is commonly used as a proxy for infection, blood is temporally variable (Doehring et al. 1985), non-specific (Bosompem et al. 2004), and dipstick limitations have not been adequately described. Some studies have used single egg counts to estimate sensitivity, specificity, and false positive rates of the dipstick test (Hall 1982; Eltoum et al., 1992; Lengeler et al., 1993; Ndyomugyenyi and Minjas 2001; van der Werf and de Vlas 2004, French et al. 2007), but these estimates may be incorrect (Eltoum et al., 1992; Ndyomugyenyi and Minjas 2001). We demonstrate here that repeated screening is feasible and should be used to improve the accuracy of prevalence and incidence estimates, to calibrate tests among sub-samples of larger populations, and to accurately diagnose *S. haematobium* infection in a clinical setting.

Materials and Methods

Study Population

All children aged 8 to 18 years who lived in Adasawase, a town in the Eastern Region of Ghana (approx. pop. 2,000), and were enrolled and present in school as of December 2007, were invited to participate in this cross-sectional study. Recruiting and data collection took place in June and July 2008. Children were recruited via the local school system and town meetings; the latter method was primarily used to contact children not attending school. A small number of local children were unenrolled ($n \approx 15$), but all declined to participate. Based on December 2007 school rosters, 484 children were aged 8 to 18 years at the time of the study; of these, 473 (97.9%) were screened at least once. A smaller number of children ($n = 255$, 52.7%) provided all three requested urine samples; only data from these children is presented here.

Consent: Institutional Review Boards

This study was approved by the Institutional Review Boards (IRBs) of Tufts University and the Noguchi Memorial Institute for Medical Research (NMIMR) and verbal assent was obtained from each child who participated. Permission to carry out the study was also obtained from the Chief of Adasawase and from the head of each school; these same individuals communicated the nature of the study to the larger community.

Study Design

There is no gold standard diagnostic test for urinary schistosomiasis (Koukounari et al., 2009). Two diagnostic tests (a dipstick test for hematuria and a filtration test for *S. haematobium* eggs)

per sample were conducted on the day that samples were collected. A single certified laboratory technician from NMIMR read all slides within four weeks of sample processing. The technician was blinded as to the identities of study participants and to the results of each participant's previous screening(s). In June 2008, all 484 children listed on school rosters were asked to provide three urine samples on three different days. In all, 255 children were screened in triplicate. To minimize disruptions to schooldays, no additional tests were conducted. In July 2008, all children in the study population were offered praziquantel (40 mg/kg) by nurses from Ghana Health Services; 404 children received praziquantel, including 240 of the 255 children who were screened in triplicate.

Urine Processing

Children were given conical tubes (50ml) for urine collection. After sample collection, urine was tested for microhematuria via a semi-quantitative dipstick test (Mott et al., 1985) (U-11 Urinalysis Reagent Strips, Mindray Co. Ltd., China) and for the presence of eggs via filtration through Nucleopore membranes (25mm diameter, 12.0μm pores). Urine was filtered for eggs as follows. 765 (3 x 255) samples were tested in total. 582 samples were shaken and 10ml of urine were drawn into a plastic syringe and then discharged through a Nucleopore membrane. For the remaining 183 samples, an experimental testing method was used in which containers were shaken and the entire volume of each sample was drawn into a syringe and discharged through a membrane. The results of using the experimental 'full-volume' method are part of a companion study and will be reported elsewhere. For the purposes of this study, it is important to note that this experimental method is slightly more sensitive than the method in which 10ml of urine are

filtered; thus, estimates of filtration test sensitivity and dipstick test specificity are really "best-case scenario" estimates.

Data Entry

Data were entered into SPSS 14.0 as total egg counts per 10ml of urine. When more then 10ml of urine was filtered, the total egg count was divided by the filtered volume and multiplied by 10. Hematuria data were first entered into SPSS 14.0 as a score (0, "negative"; 0.5+, "trace microhematuria"; 1+ through 3+, "microhematuria"; 4+, "5 to 10 erythrocytes per 1ml urine"; and 5+, "50 erythrocytes per 1ml urine"), but were reduced to binary data (presence/absence) for this analysis. A sample scoring 0.5+ or higher was considered 'positive' for hematuria.

Sensitivity and Specificity of Dipstick Test

The sensitivity and specificity of a diagnostic test can be calculated and used to describe test performance. In assessing sensitivity, 'true positive' infection status was defined as presenting with eggs at least once. By definition, the specificity of the filtration test for eggs is 100%. The specificity of the dipstick test for hematuria was calculated by defining a 'true negative' as a person who never presents with eggs and a 'false positive' as a person who presents with hematuria but never with eggs. The absence of eggs in three urine samples does not guarantee that a person is truly uninfected. Had individuals in this study been screened more than three times in June 2008, it is likely that a greater number of egg-positive (true positive) individuals would have been identified. Thus, the sensitivity of the filtration test is likely somewhat overestimated here, and the specificity of the dipstick test is probably underestimated.

Prevalence Estimates

The mean prevalence given single screening was calculated as the arithmetic mean prevalence based on the results of three different single-screenings. The mean prevalence given duplicate screening was calculated as the arithmetic mean based on all unique combinations of duplicate screenings of the population. The minimum prevalence was the lowest measured prevalence given all combinations of single or duplicate screenings. The maximum prevalence was the highest measured prevalence given all combinations of single or duplicate screenings.

Results

Description of Study Population

Study participants were recruited and urine samples were collected in June 2008. A total of 121 girls aged 8 to 17 years and 134 boys aged 8 to 18 years were screened three times in June. Study participants were not asked to self-report symptoms of urogenital schistosomiasis (dysuria, macrohematuria); no other data were collected about clinical symptoms. All children who were invited to participate in the study agreed to participate if they were present. Altogether, hematuria data are missing for four samples and filtration data are missing for nineteen samples due to technical mistakes; no sample was purposely excluded.

Infection Prevalence and Intensity Given Multiple Screenings

The following analysis is based on three collected urine samples per child. The urine volume in 6 samples was less than 10ml (range 6 – 8ml), but these samples are included and normalized to present data as eggs/10ml urine. Infection intensity (light: < 50 eggs/10ml urine, heavy: ≥ 50 eggs/10ml urine) was based on the maximum egg count of each child given three screenings

(Table 1). Compared with girls, boys had a higher prevalence of infection, a greater percentage of heavy infections, and marginally higher median 'maximum' egg counts (median 'maximum egg count' of lightly infected children: girls, 3.0 eggs/10ml; boys, 5.5 eggs/10ml, median 'maximum egg count' of heavily infected children: girls, 115 eggs/10ml; boys, 120 eggs/10ml). Twenty-three of 39 lightly-infected girls and 25 of 58 lightly-infected boys were egg-positive only once. In contrast, 1 of 5 girls and 1 of 10 boys with heavy infections were egg-positive only once (Table 1).

Table 1: Number of times each child tested positive for S. haematobium eggs and/or hematuria during three screenings, stratified by sex; number of times each child tested positive for S. haematobium eggs stratified by infection intensity.

		Number of Times each Child tested Positive for *S. haematobium* Eggs									
		0		1		2		3		Total	
		Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
Number of Times each Child tested Positive for Haematuria	0	64	53	10	14	3	5	1	1	78	73
	1	11	9	3	3	2	4	2	3	18	19
	2	1	3	5	5	2	5	3	3	11	16
	3	1	1	6	4	3	11	4	10	14	26
Infection Intensity Classification*	Neg.	77	66	0	0	0	0	0	0	77	66
	Light	0	0	23	25	8	22	8	11	39	58
	Heavy	0	0	1	1	2	3	2	6	5	10
Total		77	66	24	26	10	25	10	17	121	134

*Infection intensity based on the maximum *S. haematobium* egg count in three urine samples.

Infection prevalence among children screened in triplicate was rigorously defined as the percentage of children presenting with *S. haematobium* eggs at least once. In all, 44 girls (36.4%) and 68 boys (50.7%) were positive. Only 4 girls and 10 boys provided three samples that all

contained eggs and blood (Table 1). The minimum prevalence estimates (Table 2) based on filtration of one urine sample per person are roughly half the estimates obtained given three

Table 2: The mean, minimum, and maximum prevalence of schistosomiasis, given one, two, or three screenings per child.

	Number of Screenings per Child					
	1		2		3	
	Girls	Boys	Girls	Boys	Girls	Boys
Mean Prevalence of Schistosomiasis*	20.4%†	31.6%†	29.5%	43.0%	36.4%††	50.7%††
Minimum Measured Prevalence of Schistosomiasis*	17.4%	22.4%	24.8%	40.3%		
Maximum Measured Prevalence of Schistosomiasis*	25.6%	36.6%	32.2%	46.3%		

*By demonstration of *S. haematobium* eggs in urine

† Statistically signficant difference, $p < 0.05$

†† Statistically signficant difference, $p < 0.05$

screenings, and the mean prevalence estimated after single screening is roughly two-thirds of the prevalence estimated by triplicate screening. In this study, some individuals first presented with eggs during the third screening, indicating that true prevalence still has not been accurately assessed. When children who presented with either eggs and/or hematuria were considered 'true positives', 57 girls (47.1%) and 81 boys (60.4%) were characterized as infected (Table 3).

Table 3: The cumulative number and percentage (in parentheses) of children (n = 121 girls, n = 134 boys) who test positive for hematuria and/or S. haematobium eggs, given testing of three urine samples; the percentage of newly-detected infections at each screening, by S. haematobium egg count, where the numerator is the number of newly-detected egg-positive children, the denominator is the number of children who have not presented with eggs prior to the indicated screening, and the percentage of positive children at a given screening is shown in parentheses.

Cumulative Number of Children Testing Positive for Hematuria and/or *S. haematobium* Eggs						
	1st Screening		2nd Screening		3rd Screening	
	Female	Male	Female	Male	Female	Male
Hematuria	26 (21.5)	39 (29.1)	36 (29.8)	57 (42.5)	43 (35.5)	61 (45.5)
S. haematobium Eggs	22 (18.2)	30 (22.4)	30 (24.8)	57 (42.5)	44 (36.4)	68 (50.7)
Hematuria and/or *S. haematobium* Eggs	34 (28.1)	49 (36.6)	45 (37.2)	73 (54.5)	57 (47.1)	81 (60.4)
Newly-Dectected Infections, by *S. haematobium* Egg Count						
S. haematobium Eggs	22/121 (18.2)	30/134 (22.4)	8/99 (8.1)	27/104 (26.0)	14/91 (15.4)	11/77 (14.3)

Sensitivity and Specificity of Filtration and Dipstick Tests

In terms of detecting *S. haematobium* infection (as opposed to related morbidity), the sensitivity of the filtration and dipstick tests and the specificity of the dipstick test are presented in Table 4. Mean sensitivities and specificities are shown. Screening each child in the study three times improved the mean sensitivity of the dipstick and filtration test by at least 22.9% percent, as compared with single screening. The specificity of the dipstick test remained high (minimum value = 80.3%), even when children were screened three times.

Table 4: Sensitivity of dipstick test for hematuria and filtration test for eggs in urine and specificity of dipstick test for hematuria, based on the definition of a 'true positive' as a child who presents with eggs at least once.

		Sensitivity (%)				Specificity (%)	
		Dipstick Test		Filtration Test		Dipstick Test	
		Girls	Boys	Girls	Boys	Girls	Boys
	1	50.0	50.0	50.0	44.1	94.8	92.4
Screening	2	47.7	57.4	47.7	70.6	90.9	87.9
	3	52.3	55.9	70.5	72.1	93.5	92.4
Arithmetic Mean		50.0	54.4	56.1	62.3	93.1	90.9
	1 and 2	61.4	67.6	68.2	83.8	88.3	83.3
Screening	1 and 3	63.6	64.7	88.6	79.4	89.6	86.4
	2 and 3	63.6	64.7	86.4	91.2	85.7	84.8
Arithmetic Mean		62.9	65.7	81.1	84.8	87.9	84.8
Screening	1, 2, and 3	68.2	70.6	100.0	100.0	83.1	80.3
Percent Improvement between Mean Single Screening and Triplicate Screening		26.7	22.9	43.9	37.7	-12.0	-13.2

Discussion

The data reported here have implications for clinical screening of individuals, implementation and assessment of control programs and control tools, and accurate estimation of true urogenital schistosomiasis prevalence worldwide. In a clinical setting, repeatedly screening patients suspected of having schistosomiasis could improve the accuracy of diagnosis and thus, management of the disease. If a control program such as selective praziquantel administration is to be carried out based on the results of dipstick tests for hematuria, a 2002 WHO recommendation, the criteria for distinguishing 'true positives' and 'true negatives' should be explicitly stated (i.e. number of screening tests, type of examination). Moreover, the policy of selective treatment should be reconsidered, given the low sensitivity of dipstick tests when used with lightly-infected patients (Feldmeier et al., 1982), as shown here. When schistosomiasis control interventions such as mass chemotherapy, infrastructure alterations (WHO 2002), or vaccines are assessed for efficacy, our data suggest that at least two screenings per individual in

a representative group should be conducted pre- and post-intervention. This is in agreement with previous studies. For example, the sensitivity of the dipstick test for blood improves as mean egg intensity increases (Feldmeier et al. 1982, Lengeler at al. 1993, Ndyomugyenyi and Minjas 2001, Stephenson et al. 1984). Savioli et al. (1990) found that two urine filtration tests per person were needed before the filtration-estimated prevalence of urinary schistosomiasis was close to the prevalence estimated via a single dipstick test. Stephenson et al. (1984) tested a sub-population of Kenyan schoolchildren (n = 244) twice, once before and once after administration of metrifonate, and found that dipstick test sensitivity (based on comparison with a single filtration test for eggs) decreased from 88% pre-treatment to 64% post-treatment, and specificity decreased from 97% to 87%. These results strongly suggest that the sensitivity and specificity of the dipstick test should be evaluated in each endemic setting prior to interpreting results, as individuals with light *S. haematobium* infections may be missed by dipstick and filtration techniques, given single-day testing. One attractive option for large-scale programs is to repeatedly screen a small, randomly selected sub-population and then estimate community-wide prevalence. We recognize that this may represent additional expense in terms of time and personnel, but it is currently the best available strategy for accurate program assessment.

Schistosomiasis is the second most prevalent parasitic disease after malaria. Gross underestimation of the number of affected people artificially reduces the attention paid to schistosomiasis, which partially explains why schistosomiasis is a Neglected Tropical Disease (NTD). The global burden of schistosomiasis was recently reassessed (Steinmann et al., 2006; Hotez and Kamath 2009), but worldwide prevalence and morbidity/mortality estimates are commonly made by extrapolating from cross-sectional studies (Ratard et al., 1992; Engels et al., 2002; van der Werf et al., 2003) in which individuals are screened once. For example, Parkin

(2008) estimated the prevalence of bladder cancer due to *S. haematobium* infection and van der Werf et al. (2003) estimated the total morbidity associated with schistosome infections in sub-Saharan Africa using such techniques. In another study, adults in Ghana were screened on one occasion via five diagnostic methods. Results were analyzed using latent class modeling to estimate the uncertainty of sensitivity and specificity for each diagnostic test (Koukounari et al. 2009). However, if participants had been screened repeatedly and 'false negatives' correctly identified as 'true positives', the estimate of filtration test sensitivity would have been lower and the specificity of other tests may have been higher. Underestimation of infection prevalence given single screening has been well-documented in the study of stool examination for *S. mansoni* eggs (Utzinger et al. 2001). Utzinger et al. (2001) also showed that examining a single stool specimen in various locations improves diagnostic test sensitivity, which is conceptually similar to filtration of an entire urine sample instead of a 10ml sub-sample.

Most infected children in this study presented with fewer than 50 eggs/10ml urine. Lightly-infected children did not always present with hematuria or eggs and, surprisingly, this was the case even for heavily-infected children (> 50 eggs/10ml urine). Thus, children cannot be characterized as uninfected after one screening. Ideally, the number of previously undetected infections would approach zero at the second and third screenings, but there is no such consistent trend in our data. Additional studies should be carried out to determine the number of screenings required before prevalence estimates are reasonably accurate; in particular, it would be valuable to study diagnostic accuracy among children recently treated with praziquantel (WHO 2000; Bosompem et al., 2004; Saathof et al., 2004; Coon 2005; Gryseels et al., 2006).

Acknowledgements

We would like to thank Mr. Michael Nii Adjei and Mr. Dickson Osabutey for their invaluable assistance with fieldwork, and the individuals in Adasawase who made this work possible: the schoolchildren, school officials, Osabarima Kwame Tia II, Hon. Alex Asamoa-Bonti, Ghana Health Services, and the Council of Elders. We thank Dr. M. Woodin for statistical assistance and Dr. J. K. Griffiths for commenting on the manuscript. We thank Mr. J. Crocker for his engineering expertise, and members of the construction team for their dedication and hard work.

References

1. Bosompem, K.M., Asigbee, J., Otchere, J., Haruna, A., Kpo, K.H., Kojima, S., 1998. Accuracy of diagnosis of urinary schistosomiasis: Comparison of parasitological and a monoclonal antibody-based dipstick method. Parasitol. Int. 47, 211-217.
2. Bosompem, K.M., Bentum, I.A., Otchere, J., Anyan, W.K., Brown, C.A., Osada, Y., Takeo, S., Kojima, S., Ohta, N., 2004. Infant schistosomiasis in Ghana: a survey in an irrigation community. Trop. Med. Int. Health. 9, 917-922.
3. Coon, D.R., 2005. Schistosomiasis: Overview of the history, biology, clinicopathology, and laboratory diagnosis. Clin. Microbiol. Newsletter. 27, 163-168.
4. Doehring, E., Vester, U., Ehrich, J.H.H., Feldmeier, H., 1985. Circadian variation of ova excretion, proteinuria, hematuria, and leukocyturia in urinary schistosomiasis. Kidney Int. 27, 667-671.
5. Eltoum, I.A., Sulaiman, S., Ismail, B.M., Ali, M.M.M., Elfatih, M., Homeida, M., 1992. Evaluation of haematuria as an indirect screening test for schistosomiasis haematobium: a population-based study in the White Nile province, Sudan. Acta Trop. 51, 151-157.
6. Engels, D., Chitsulo, L., Montresor, A., Savioli, L., 2002. The global epidemiological situation of schistosomiasis and new approaches to control and research. Acta Trop. 82, 139-146.
7. Feldmeier, H., Bienzle, U., Dietrich, M., Sievertsen, H.J., 1979. Combination of a viability test and a quantification method for Schistosoma haematobium eggs (filtration--trypan blue-staining-technique). Tropenmed. Parasitol. 30, 417-422.
8. Feldmeier, H., Doehring, E., Daffala, A.A., Omer, A.H.S., Dietrich, M., 1982. Efficacy of metrifonate in urinary schistosomiasis: comparison of reduction of *Schistosoma*

haematobium and *S. mansoni* eggs. Am. J. Trop. Med. Hyg. 31, 1188-1194.

9. Feldmeier, H., Doehring, E., Daffalla, A.A., 1982. Simultaneous use of a sensitive filtration technique and reagent strips in urinary schistosomiasis. Trans. R. Soc. Trop. Med. Hyg. 76, 416-421.
10. Feldmeier, H., Poggensee, G., 1993. Diagnostic techniques in schistosomiasis control. A review. Acta Trop. 52, 205-220.
11. French, M.D., Rollinson, D., Basáñez, M.G., Mgeni, A.F., Khamis, I., Stothard, J.R., 2007. School-based control of urinary schistosomiasis on Zanzibar, Tanzania: monitoring micro-haematuria with reagent strips as a rapid urological assessment. J. Pediatr. Urol. 3, 364-368.
12. Gryseels, B., Polman, K., Clerinx, J., Kestens, L., 2006. Human schistosomiasis. Lancet. 368, 1106-1118.
13. Hall, A., 1982. Intestinal helminths of man: the interpretation of egg counts. Parasitology. 85, 605-613.
14. Hotez, P.J., Kamath, A., 2009. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. PLoS Negl. Trop. Dis. 3, e412.
15. Koukounari, A., Webster, J.P., Donnelly, C.A., Bray, B.C., Naples, J., Bosompem, K., Shiff, C., 2009. Sensitivities and Specificities of Diagnostic Tests and Infection Prevalence of Schistosoma haematobium Estimated from Data on Adults in Villages Northwest of Accra, Ghana. Am. J. Trop. Med. Hyg. 80, 435-441.
16. Lengeler, C., Mshinda, H., Morona, D., deSavigny, D., 1993. Urinary schistosomiasis: Testing with urine filtration and reagent sticks for haematuria provides a comparable prevalence estimate. Acta Trop. 53, 39-50.
17. Mafiana, C.F., Ekpo, U.F., Ojo, D.A., 2003. Urinary schistosomiasis in preschool children in

settlements around Oyan Reservoir in Ogun State, Nigeria: implications for control. Trop. Med. Int. Health. 8, 78-82.

18. McCullough, F.S., Bradley, D.J., 1973. Egg output stability and the epidemiology of Schistosoma haematobium. I. Variation and stability in Schistosoma haematobium egg counts. Trans. R. Soc. Trop. Med. Hyg. 67, 475-490.
19. Ndyomugyenyi, R., Minjas, J.N., 2001. Urinary schistosomiasis in schoolchildren in Dar-es-Salaam, Tanzania, and the factors influencing its transmission. Ann. Trop. Med. Parasitol. 95, 697-706.
20. Opara, K.N., Udoidung, N.I., Ukpong, I.G., 2007. Genitourinary schistosomiasis among pre-primary schoolchildren in a rural community within the Cross River Basin, Nigeria. J. Helminthol. 81, 393-397.
21. Parkin, D.M., 2008. The global burden of urinary bladder cancer. Scand. J. Urol. Nephrol. 42, 12-20.
22. Peters, P.A., Mahmoud, A.A.F., Warren, K.S., Ouma, J.H., Siongok, T.K.A., 1976. Field studies of a rapid, accurate means of quantifying *Schistosoma haematobium* eggs in urine samples. Bull. WHO. 54, 159-162.
23. Peters, P.A., Warren, K.S., Mahmoud, A.A., 1976. Rapid, accurate quantification of schistosome eggs via Nuclepore filters. J. Parasitol. 62, 154-155.
24. Ratard, R.C., Kouemeni, L.E., Bessala, M.M., Ndamkou, C.N., 1992. Estimation of the number of cases of schistosomiasis in a country: the example of Cameroon. Trans. R. Soc. Trop. Med. Hyg. 86, 274-276.
25. Saathoff, E., Olsen, A., Magnussen, P., Kvalsvig, J.D., Becker, W., Appleton, C.C., 2004. Patterns of *Schistosoma haematobium* infection, impact of praziquantel treatment and re-

infection after treatment in a cohort of schoolchildren from rural KwaZulu-Natal/South Africa. BMC Infect. Dis. 4, 40.

26. Savioli, L., Hatz, C., Dixon, H., Kisumku, U.M., Mott, K.E., 1990. Control of morbidity due to *Schistosoma haematobium* on Pemba Island: egg excretion and hematuria as indicators of infection. Am. J. Trop. Med. Hyg. 43, 289-295.
27. Steinmann, P., Keiser, J., Bos, R., Tanner, M., Utzinger, J., 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect. Dis. 6, 411-425.
28. Stephenson, L.S., Latham, M.C., Kinoti, S.N., Oduori, M.L., 1984. Sensitivity and specificity of reagent strips in screening of Kenyan children for *Schistosoma haematobium* infection. Am. J. Trop. Med. Hyg. 33, 862-871.
29. Utzinger, J., Booth, M., N'Goran, E.K., Müller, I., Tanner, M., Lengeler, C., 2001. Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. Parasitology. 122, 537-544.
30. van der Werf, M.J., de Vlas, S.J., Brooker, S., Looman, C.W.N., Nagelkerke, N.J.D., Habbema, J.D.F., Engels, D., 2003. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta Trop. 86, 125-139.
31. Warren, K.S., Siongok, T.K.A., Houser, H.B., Ouma, J.H., Peters, P.A., 1978. Quantification of infection with *Schistosoma haematobium* in relation to epidemiology and selective population chemotherapy. I. Minimal number of daily egg counts in urine necessary to establish intensity of infection. J. Infect. Dis. 138, 849-855.
32. World Health Organization [WHO], 2001. Report of the WHO informal consultation on schistosomiasis in low transmission areas: control strategies and criteria for elimination.

WHO/CDS/CPE/SIP/2001.1. World Health Organization, London, England.

33. World Health Organization, 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: Report of a WHO expert committee. Tech. Rep. Series 912. World Health Organization, Geneva, Switzerland.

34. World Health Organization, 2005. Report on Schistosomiasis, Scientific Working Group on Schistosomiasis. Special Programme for Research and Training in Tropical Diseases (TDR), TDR/SWG/07. World Health Organization, Geneva, Switzerland.

CHAPTER 4: A NOVEL COMMUNITY-BASED WATER RECREATION AREA FOR SCHISTOSOMIASIS CONTROL IN RURAL GHANA

Abstract

Primary prevention of schistosome infection has received little attention to date. In this paper we describe a novel water recreation area (WRA) to reduce *Schistosoma haematobium* infection rates in Adasawase, Ghana. Urogenital schistosomiasis is a waterborne parasitic disease that affects over 100 million people worldwide, primarily children in the rural tropics. The disease is contracted via dermal contact with contaminated water. Mass chemotherapy is presently used to control morbidity, but chemotherapy does not confer immunity and reinfection has severe health impacts. In 2008, over 50% of boys and 36% of girls in Adasawase had *S. haematobium* eggs in their urine. Recreational contact with water was the primary transmission route. In collaboration with community members, a novel water recreation area (WRA) was constructed. The WRA is groundwater and rainwater fed and serves more than 100 children at any given time. It was constructed from local materials and labor, designed to last more than 30 years, and minimizes exposure to *S. haematobium*. One year after construction, the annual incidence of *S. haematobium* infection dropped from 17.4% (girls) and 26.9% (boys) in 2009 to 2.2% and 8.4% in 2010, respectively. These promising results suggest that WRAs may be useful for controlling schistosome transmission.

Introduction

Schistosomiasis is a group of diseases caused by blood flukes in the genus *Schistosoma*. Schistosomiasis is endemic in 76 countries (Engels et al. 2002). Urogenital schistosomiasis is caused by *Schistosoma haematobium* and affects an estimated 112 million people worldwide (King 2005, van der Werf et al. 2003). Collectively, Kenya, Ghana, Mozambique, Tanzania and Nigeria account for over fifty percent of the morbidity associated with the disease (van der Werf et al. 2003). Urogenital schistosomiasis has been reported in Ghana since 1895 (Doumenge et al. 1987) and is the focus of the pilot intervention study described here.

Urogenital schistosomiasis is spread through dermal contact with tropical fresh water bodies that harbor the parasite and its intermediate host, *Bulinus* snails. The life cycle is perpetuated when an infected human host urinates directly into a surface water body and releases *S. haematobium* eggs into the environment. In Adasawase, Ghana, children typically contract schistosomiasis in a local river where they play, bathe, and collect water.

Mass treatment of a population with praziquantel, the drug of choice, can be highly beneficial to schistosome-infected individuals, but can strain local health care systems (WHO 2006). Despite short-term improvements in morbidity following treatment with praziquantel (Engels et al. 2002), it is generally agreed that chemotherapy as a single strategy is rarely sustainable (Gryseels et al. 2006, Tucker 1983, Utzinger et al. 2003). Primary prevention of schistosomiasis is preferable. Several researchers have argued that areas of safe water contact are needed (Kloetzel 1992, and El-Katsha 2002). In theory, schistosomiasis in rural parts of Ghana could be effectively prevented if people had access to and used only uncontaminated water to meet their needs.

Recreational water contact is a known risk factor for schistosomiasis in endemic areas (Lima e Costa et al. 1987, Tucker 1983, Gazzinelli et al. 2001, Friedman et al. 2001, Opara et al. 2007, Ndyomugyenyi and Minjas 2001). Recreational water use often yields a relatively large surface area of skin exposed to water for prolonged periods of time (Ndyomugyenyi and Minjas 2001, Oladejo and Ofoezie 2006). No studies have been found in which researchers have examined the effects of infrastructure targeted at reducing recreational exposure. A number of papers describe schistosomiasis and recreation (Gazzinelli et al. 2001, Kloos et al. 1986, Lima e Costa et al. 1987, Mafiana et al. 2003, Ndyomugyenyi and Minjas 2001, Opara et al. 2007), but only one (Tucker 1983) specifically calls for interventions to be designed that address recreational exposure. A larger group of papers discuss the need for water, sanitation, and education, but do not mention recreational water use (Chitsulo et al. 2000, Clennon et al. 2004, Engels et al. 2002, Kabatereine et al. 2005, King et al. 2004, King et al. 2006, Lancet 2004, Nsowah-Nuamah et al. 2004, Polderman 1984, Utzinger et al. 2003, WHO 2000).

To determine whether avoidance of recreational exposure to infected river water could reduce schistosome infection in rural Ghana, a water recreation area (WRA) was selected as a potential primary prevention technique. Adasawase was selected as the intervention community based on (a) the high cross-sectional prevalence of urogenital schistosomiasis in 2008 (36.4% of girls, 50.7% of boys), (b) an invitation from the Chief of Adasawase to work in the community, and (c) the small size of the town (approximate population 2000). After establishing baseline infection prevalence, the Chief and Council of Elders considered a number of options commonly used to control schistosomiasis morbidity, including regular mass treatment with praziquantel and health and hygiene education. The idea of the WRA was also presented. Adasawase authorities chose to focus on the WRA. This was a community-based approach in which local

demand was a critical factor in selecting the appropriate intervention; this is analogous to the approach taken by the South African Department of Water Affairs and Forestry (2002) on sanitation provision. The WRA was designed to effectively and sustainably reduce infection with schistosomes and to be replicable in similar settings. This involved consideration of many constraints, including: WRA site location within the town, local construction methods and expertise, availability of materials, costs of operation and maintenance, and other demands on water resources. The WRA consists of a concrete pool with shallow and deep sections and a latrine; it is filled by a rainwater collection system and by two pre-existing hand pumped boreholes. To assess the structure's effectiveness in lowering infection incidence, children were screened for S. haematobium eggs before and after the WRA was opened for use; behavior was characterized via interviews and direct observation.

Materials and Methods

Construction Methods

Design of the WRA was based on literature describing appropriate technology for community-level operations and maintenance (Cairncross and Feachem 1993, Schulz and Okun 1984, DWAF 2004), the local availability and cost of materials, and the collective experience of the implementation team and community members (Hopkins et al. 2004). In particular, input was collected from the Chief of Adasawase, the Council of Elders, community members including schoolchildren, and particularly the community members on the construction team. Members of the construction team were hired for their demonstrated experience and knowledge of construction methods.

Selection of Water Recreation Area Site

The location of the water recreation area was chosen based on (a) proximity to two groundwater wells, (b) availability of open land, as verified by the Chief, (c) slope of the land for drainage, and (d) location between the town center and the previous water contact site at Tini River (Figure 1). The aforementioned groundwater wells with handpumps have been in use for approximately 25 years. The location of the WRA between the town and the river was selected in order to maximize the likelihood of behavior change (i.e. actually using the WRA) among schoolchildren.

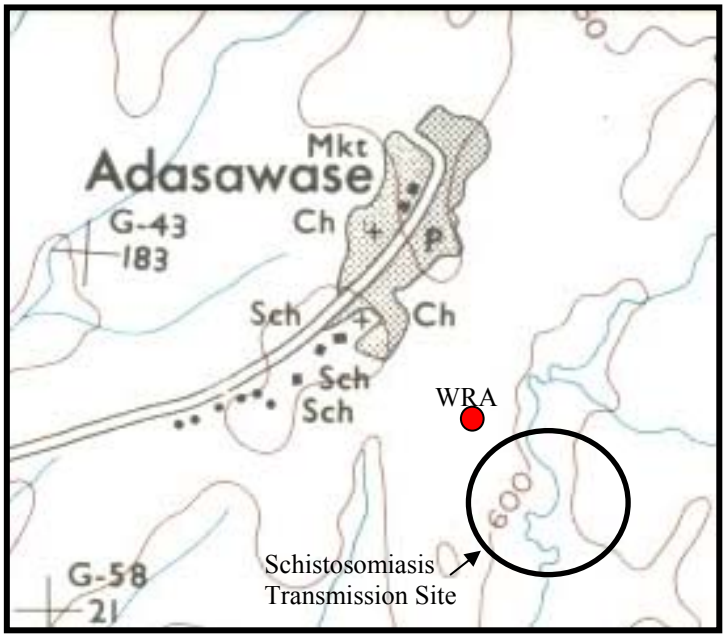


Figure 1: Map of Adasawase showing the approximate location of the water recreation area (WRA), the two primary schools and one junior high school (Sch), two churches (Ch), the Chief's palace (P), and the main market (Mkt) (Harvard Map Collection, Ghana, 1965).

Water Recreation Area Site Preparation

Preparation of the construction site involved leveling the ground (75 m^3 of soil) and excavating the soil (42 m^3) that would be replaced by the WRA. To further enhance the potential for WRA translation to similar settings, this was accomplished solely with manual labor (Figure 2).

Figure 2: Manual excavation of 42 m^3 of soil in preparation for construction of the WRA; photograph taken June 2008.

Labor for site preparation represents a community contribution to the overall cost of construction; at 750 person-hours, the contribution was substantial. In Adasawase, adult community members are normally expected to contribute service hours to projects that benefit the town (e.g. health clinic construction, road maintenance). The community members who worked on this project chose to do so in partial fulfillment of this service requirement. The project was deemed by the Chief and Council of Elders to be a public good, based on projections that it would help reduce schistosomiasis transmission and would be a resource for all local children.

Water Recreation Area Design

A WRA built with local labor and expertise out of local materials was chosen preferentially over installation of a prefabricated pool or hiring of an outside construction company. The pool was split into a deep and shallow ends designed for water depths of up to 1.22 m and 0.30 m respectively, to accommodate children of a broad range of ages. The depths were chosen based on the depth of the river swimming site, safety, and the preferences of schoolchildren.

The pool was sized based on a variety of constraints. It had to be sufficiently large to accommodate up to 100 children at a time. Simultaneously, it had to be sufficiently small to contain costs and minimize water use. Sizing the pool for routine flushing and draining was a key factor, as this eliminated the need for chemical treatment of pool water. An attempt was made to maximize the surface area to volume ratio while still keeping the pool rectangular in shape for ease of construction. The dimensions of various pool sections, and the required concrete and rebar materials are listed in Table 1.

Table 1: Details of the components of the Water Recreation Area (WRA), with accompanying specifications.

Component	Details	Value	Units
Shallow end	Interior Dimensions	5.3 x 2.3 x 0.6	m
	Volume Full	3.6	m^3
Deep end	Interior Dimensions	5.3 x 3.8 x 1.4	m
	Volume Full	22	m^3
Concrete	Footings	0.1	m^3
	Walls	2.6	m^3
	Slabs	3.3	m^3
	Skirt	2.8	m^3
	TOTAL	9.5	m^3
Rebar	Total Length	600	m
	Size (Diameter)	1.3	cm
	Vertical Spacing in Walls	0.15	m
	Grid Spacing in Floor Slabs	0.46	m

The pool is made from durable materials that do not require significant maintenance. The concrete mix was designed for high strength while still being workable without power tools. The recreation area and surrounding area were fitted with terraces and proper sloping for drainage to prevent erosion (Figure 3). The rainwater collection system is automatic and contains an overflow outlet so that during a rain event, no adjustment to valves is necessary. These design features were chosen to simplify operations and maintenance and to promote sustained use without burdening the community. The WRA was rendered in an aesthetically pleasing manner to encourage use by children; for example, the pool was waterproofed with blue paint, curved concrete walking paths were poured, and flowering plants border the area.

The unintended increase of other infectious diseases was considered. To avoid creating a breeding area for mosquitoes, the pool is covered during non-use or flushed at least once every three to four days and covered when not in use.

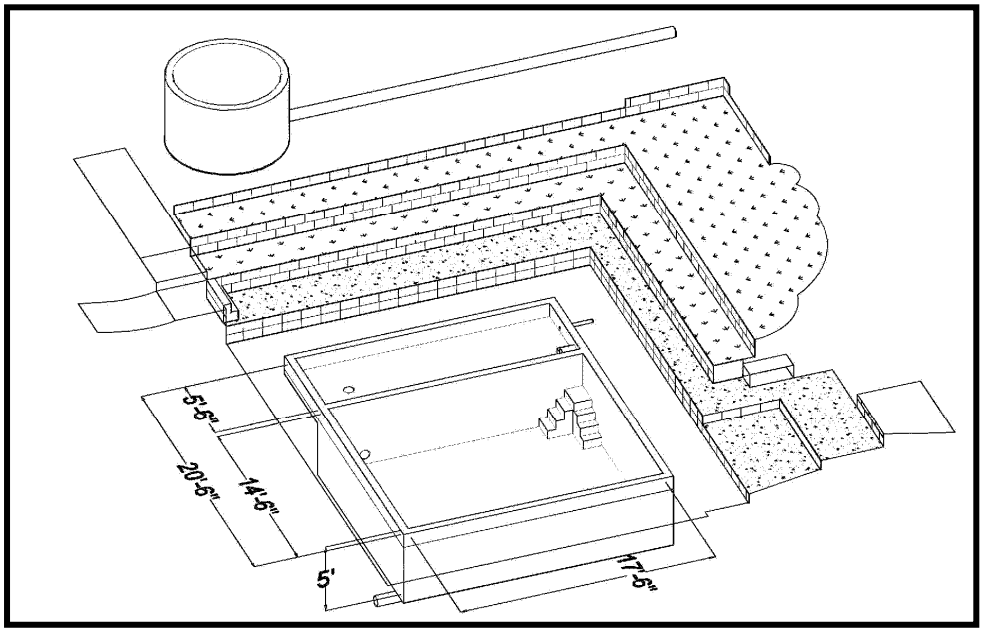


Figure 3: Schematic of the water recreation area (WRA) in Adasawase, Ghana. Erosion-control terraces, a 7.0 m^3 rainwater collection tank, and walking paths are shown; the latrine, tow borehole wells, and most components of the rainwater collection system are located outside the scope of this figure.

Local Availability of Construction Materials

Available materials included 1.27 cm iron rod (rebar), cement, 1.91 cm crushed stone, "quarry dust" (bluestone sand), and low-silt sand from a riverbank deposit. Hollow blocks were commissioned for construction of the walls of the water recreation area, but the final blocks were weak because they were made with poor quality sand and had been dried in the sun instead of cured under damp conditions. The final block dimensions were incorrect (31.12 cm x 20.32 cm x 10.16 cm instead of 30.48 cm x 20.32 cm x 10.16 cm), causing significant delays in construction and considerable additional expense. The additional expense resulted from the use of unexpected

additional labor and materials (sand, gravel, cement, rebar) when both cells in each block had to be filled with reinforced concrete to render the walls of the pool structurally sound (Figure 4). The joints of polyvinyl chloride (PVC) pipes did not always fit together. Joints frequently were softened over open flame before they were glued.

Figure 4: A hollow block construction method was used in which cells of the blocks were filled with concrete and reinforced with rebar (iron rod).

Local Knowledge and Capacity Building

Two senior and two junior masons were hired in addition to four general laborers; these individuals, along with one translator from a nearby community, comprised the Ghanaian members of the construction teams, which operated from June – August 2008 and June – August 2009. The construction team, especially the senior masons, provided context for the project by supplying knowledge of local environmental conditions and appropriate materials and construction methods (Hopkins et al. 2004). For example, erosion in Adasawase can render buildings structurally unsound and walls may cave; therefore, concrete and earthen terraces were

constructed on the hill slope near the WRA to prevent erosion and to control overland flow of rainwater during heavy precipitation events. Also, to minimize the opportunity for erosion and vandalism to damage PVC water pipes buried in the subsurface, the pipes were buried between 0.33 m and 1.0 m deep. Finally, Ghanaian team members were familiar with cyclic, seasonal changes in the geomorphology of nearby drainage canals and the local river and were able to provide information about seasonal water flow, sediment load, and the corresponding changes in drainage canal locations and depths. The construction team selected the depth and location of water inflow and outflow pipes that connect to the WRA to prevent them from being exposed and damaged or removed.

A hollow-block method was selected for construction of the pool walls. Milled lumber to create forms for poured concrete slabs was not available near Adasawase and is unlikely to be available or inexpensive in other rural schistosomiasis-endemic sites. A hollow-block construction method allowed all members of the construction team to apply local construction techniques and translates easily to similar settings. It also makes possible reinforcement of the pool walls with 1.27 cm iron rod (rebar). This strengthening technique was employed in the WRA.

Appropriate and Sustainable use of Materials and Community Resources

Adasawase is predominantly a subsistence farming community. Cash for electricity and chemicals for the pool is not available, and chemical treatment of the water was not considered the most environmentally sensitive option for pool maintenance. Instead, groundwater from a pair of handpumps located approximately 25 m from the pool is used to supply about 50% of the pool water. The approximate pumping rate is 20L/min and only one pump is connected to the

pool at any given time, leaving the second pump available for domestic water collection. The remainder of the pool water is obtained from a rainwater collection system. Three roof sections (approximately 5 m by 12 m) were guttered and connected to the WRA via 2.54 cm PVC piping laid underground; the expectation is that the gutters will collect approximately 250 m3 of rainwater per year. The pool was situated so as to utilize pre-existing natural drainage systems for periodic cleaning purposes. Gravity is sufficient to drain and re-fill the pool with the rainwater collection system. In the dry season, the pool remains empty so as not to deplete groundwater that is valuable for meeting domestic needs. In the dry season (February – April), the local swimming area at Tini River is too shallow for recreational contact with water; thus, children do not swim there then.

Assessment of WRA Impact on Schistosomiasis Incidence

Study Population

In 2009, all children over 6 years of age who were enrolled in school in Adasawase were invited to participate in this longitudinal study. Recruiting and data collection took place between June of 2009 and August 2010 via the local school system. Based on official school rosters, 472 children were over 6 years of age at the time of the study; of these, 437 (92.6%) were in school on at least one screening day and all agreed to participate. Attendance at school in these age groups is compulsory and generally quite complete. A smaller number of children (n = 210) provided at least three urine samples in June/July 2009, as well as three or more urine samples in June/July 2010. Only data from children with triplicate samples are presented here.

Consent: Institutional Review Boards

This study was approved by the Institutional Review Boards (IRBs) of Tufts University and the Noguchi Memorial Institute for Medical Research (NMIMR) and verbal assent was obtained from each child who participated. Permission to carry out the study was also obtained from the Chief of Adasawase and from the head of each school; these same individuals communicated the nature of the study to the larger community.

Study Design

In June/July 2009, all children who were at least six years old and attended school in Adasawase were invited to provide urine samples for schistosomiasis screening. School enrollment is compulsory and quite complete. Each was asked to provide one sample per day on three different days between 1000 and 1400 hours. Two diagnostic tests—a dipstick test for haematuria and a filtration test for *S. haematobium* eggs—were conducted on each sample on the day that samples were collected. A single certified laboratory technician from NMIMR read all slides within two weeks of sample processing. The technician was blinded as to the identities of study participants and to the results of each participant's previous screenings. This process was repeated in June/July of 2010 with the same individuals. Individuals who did not provide at least three samples in 2010 are not included in the analysis presented here.

Determination of Infection Status

Children were given 50ml plastic containers for urine collection. After sample collection, urine was tested for microhaematuria via a semi-quantitative dipstick test (Mott et al. 1985) (U-11 Urinalysis Reagent Strips, Mindray Co. Ltd., China) and for the presence of eggs via filtration

through Nucleopore membranes (25mm diameter, 12.0μm pores). Urine was filtered for eggs as follows: urine containers were shaken and 10ml of urine were drawn into a plastic syringe and then discharged through a Nucleopore membrane. Membranes were placed on glass slides and eggs were counted at 40x power.

To determine disease status, a "true positive" individual was defined as a person who presented with eggs at least once. However, the absence of eggs in three urine samples does not guarantee that a person is uninfected. Data were entered into SPSS 14.0 as total egg counts per 10ml of urine and were then reduced to binary data (presence/absence). Haematuria data were first entered into SPSS 14.0 as a score, but were reduced to binary data (presence/absence) for analysis.

Results and Discussion

Construction of concrete structural elements of the pool lasted approximately eight weeks and took place during June, July, and August of 2008. Between June and early August 2009, waterproofing was completed and other components of the WRA, such as the terracing, latrine, and rainwater collection system were added. Community overseers and maintenance workers were trained, and upon completion of the WRA, children were encouraged by the local Assemblyman to use the water collection facilities and to recreate at the WRA instead of at the Tini River. The Assemblyman undertook a word-of-mouth campaign over the course of several weeks to inform children and their parents about the nature of the pool and its purpose as a form of schistosomiasis-transmission control.

Recreational Water Contact at Tini River

Between July and November of 2009, an observer was hired from Adasawase to monitor behavior at Tini River (Figure 5). He was stationed at the edge of the river from 7am to 5pm, 7 days per week, and was asked to record the names, ages, school affiliations, types and durations of water contact activities, and time of day that each child visited the river. Data show that of 190 girls and 214 boys in Adasawase who submitted urine samples for screening in 2010, 17 girls and 42 boys visited the river at least once during this period. Among children who visited the river for any kind of water activity, incidence of infection was 5.9% of girls and 7.1% of boys. Among children who did not visit the river during this time, incidence was 1.1% of girls and 5.1% of boys. It is important to note that even among children who did visit Tini River, the cumulative contact time over the 4.5 month period was minimal. Of the 17 girls who visited the river, mean contact time was 4.49 hours per 4.5 months (range: 0.17 to 11.42hrs); of the 42 boys, mean contact time was 3.37hrs per 4.5 months (range: 0.08 to 13.87hrs). "Risky" contact was defined as swimming or bathing in Tini River; on average, girls had 0.78hrs (range: 0.00 to 3.00hrs) of 'risky' contact and boys had an average of 0.89hrs (range: 0.00 to 3.78hrs) of 'risky' contact during the 4.5-month period.

Figure 5: Children recreating at Tini River in 2008, prior to the opening of the WRA.

The Water Recreation Area

The recreation area is open for use three to four days per week in order to periodically flush the pool water and maintain water quality in the absence of chemicals and filtration. This management scheme was selected by the community as a feasible and sustainable way of meeting the needs of the end users (i.e. schoolchildren). The WRA was operational between August 2009 and August 2010 under town management. Because chemicals and electricity are not necessary for operation, the community has all the resources required for use: water and manual labor. Children of all ages play in the shallow end on a regular basis, and when a rain event occurs, the deep end is also opened (Figure 6). In June 2010, the WRA was visited by our study team to assess wear and to consider design components that required improvement. The drainage system of the pool in the deep end had to be unearthed due to clogging; the problem was effectively remedied by inserting a solid 10.16 cm PVC solid cap into the drain and drilling into it a series of holes, thereby allowing the cap to act as a strainer for organic material. This was the only aspect of the pool that required redesign.

Figure 6: Children recreating at the WRA in August 2009.

Operations and Maintenance

An Operations and Maintenance (O&M) plan was designed in collaboration with the community. The objective of the plan was to maintain pool water quality at an acceptable level as defined by the community. Construction methods and design features were chosen so that the facility could be operated, cleaned, and repaired with local knowledge and expertise. A high-strength concrete mix with a synthetic additive allowing for lower water content and porosity was selected for construction of the pool. The concrete was over-engineered for structural stability to obviate the need for frequent repairs. All pipes were made of PVC and the systems were buried to protect the PVC from ultraviolet degradation and also from accidental or intentional anthropogenic damage. A number of community members know where the pipes are laid and are capable of repairing them. The WRA has no 'black box' parts: the rainwater collection system is visible and intuitive, connections to handpumps are visible, drains are visible and accessible, and the latrine was constructed via a method common to the community. One paid, part-time individual oversees the general cleanliness of the WRA, safety, and water levels in the pool.

Changes in S. haematobium Infection Incidence

Cross-sectional data collected in 2008 show that at least 36% of girl children and 50% of boy children were infected with *S. haematobium*, based on egg counts. These prevalence estimates were made after triplicate screening of most of the children in the population. Following screening, 416 of 473 (87.9%) schoolchildren were treated with praziquantel by Ghana Health Services; 203 of 255 previously-positive children were retested once for eggs and blood after praziquantel administration and all but one child cleared the parasite.

In June 2009, all schoolchildren in Adasawase again were invited to submit urine samples for schistosomiasis screening. Most children were screened four times each. Among children who were screened four times in 2009, annual incidence was estimated to be 26% (n = 37/141) among girls and 30% (46/156) among boys. Thus, ongoing disease transmission was demonstrated to occur between June 2008 and June 2009. In July 2009, Ghana Health Services professionals again offered treatment to all children and 419 of 472 (88.8%) schoolchildren received praziquantel. Of those treated, 9 children presented with eggs and/or blood during follow-up testing conducted several weeks later.

Children were rescreened in June and July 2010. Only data from children who were screened three times in both 2009 and 2010 are shown in Figure 7. Of 92 girls screened in triplicate in both years, 76 were consistently negative for eggs in urine, 14 were positive in 2009 and did not become reinfected, and 2 were positive during both 2009 and 2010 screening efforts. Of the 119 boys screening in triplicate, 79 were always egg-negative, 30 were positive in 2009 and did not become reinfected, 2 were consistently positive, and only 8 boys were egg-negative in 2009 and egg-positive in 2010.

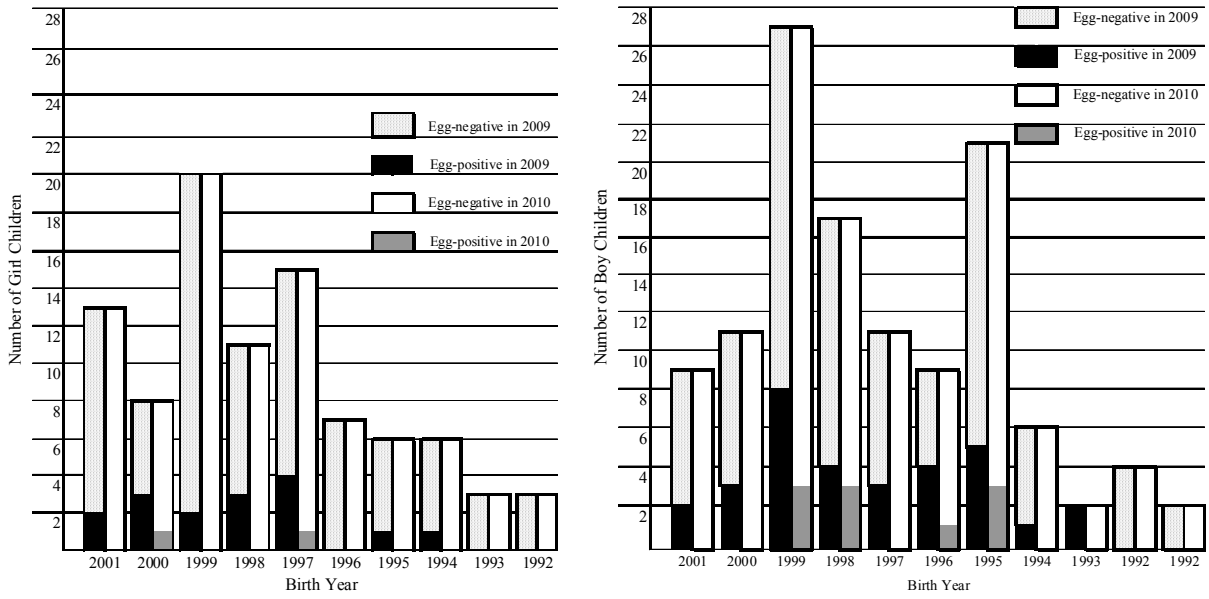


Figure 7: *S. haematobium* infection status (by egg count) for Adasawase children by birth year; all children submitted at least three urine samples in both 2009 and 2010.

Annual incidence among girls screened in quadruplicate in 2009 was 16.4% (n = 23/140); among boys, it was 25% (n = 38/154). Annual incidence among girls screened in quadruplicate in 2010 was 2.6% (n = 3/116); among boys, it was 7.4% (n = 11/149). Differences in the denominator reflect the slight differences in cohort composition depending on the number of screenings each child experienced in a given year. Children who were screened two, three or four times were similar to each other in terms of incidence of infection (data not shown). Children who were screened only once were more likely to be infected than their peers. However, a number of other factors were different for this small number of children, and a more nuanced statistical analysis is necessary before more definitive conclusions can be drawn.

Conclusions

Annual incidence of infection with *S. haematobium* decreased dramatically between 2009 and 2010 in Adasawase with the introduction of the WRA, when compared with incidence between 2008 and 2009 (with no WRA present). To our knowledge, the presence of the WRA is the only community-wide factor that changed during this time period that could affect incidence to this extent. While the data cannot demonstrate causation, they strongly suggest that the WRA contributed significantly to a reduction in *S. haematobium* infection incidence. Our approach should be repeated in other rural communities where *S. haematobium* is endemic to determine whether the results are reproducible.

In general, primary prevention of schistosomiasis receives very little attention in the literature, with some notable exceptions (King 2010, King 2010b, King et al. 2006), and in particular, there is very little focus on preventing recreational contact with contaminated surface water. In Japan, diminished river contact has been shown to decrease reinfection with and severity of disease caused by schistosome worms (Minai et al. 2003) since, in the absence of acquired immunity, parasite burden is directly proportional to the frequency and duration of contact with infected waters. In a more recent paper, Wang et al. (2009) found that in China, the use of infrastructure plus an integrated package of disease control tools (education, chemotherapy, alternative agriculture methods, control of animal reservoirs, etc.) was effective in reducing *S. japonicum* transmission. Chemotherapy had previously been used, but had proved ineffective at reducing parasite transmission below an unyielding endemic level. To our knowledge, ours is the first paper discussing the use of infrastructure to alter recreational water contact behavior in the context of schistosomiasis.

Our work has several important limitations. First, it is recognized that lack of disinfection (chlorine) may permit some pathogenic microorganisms to grow in the WRA; however, given the heavy use of the contaminated Tini River swimming area, we believe that the pool represents a net improvement over the quality of water at the river, which is functionally stagnant for much of the year. Further studies are needed to confirm that water quality at the WRA is consistently free of unacceptably high levels of bacteria or other undesirable organisms. Second, there are confounding variables such as school attendance, children's house locations, and frequency of contact with the river, that warrant further in-depth statistical analysis to elucidate precisely which risk factors contribute to *S. haematobium* infection. Another key issue is the impact of regular treatment with praziquantel; water contact has been reduced in the presence of the WRA, but regular drug administration is a critical variable to examine in future studies. Future refinements in the analysis of these outcomes are warranted. These shall form the basis of future publications. Our intent here was to provide detail concerning the construction of the WRA and to present data that suggest efficacy associated with its use. Given the limited nature of this pilot study, and the very encouraging decrease in infection incidence, the WRA appears to be a new potential tool for integrated schistosomiasis control.

References

1. Cairncross, S. and R. Feachem, 1993. *Environmental Health Engineering in the Tropics*. New York, John Wiley & Sons.
2. Chitsulo, L., D. Engels, A. Montresor and L. Savioli, 2000. The global status of schistosomiasis and its control. *Acta Tropica* 77:41-51.
3. Clennon, J. A., C. H. King, E. M. Muchiri, H. C. Kariuki, J. H. Ouma, P. Mungai and U. Kitron, 2004. Spatial patterns of urinary schistosomiasis infection in a highly endemic area of coastal Kenya. *The American Journal of Tropical Medicine and Hygiene* 70:443.
4. Department of Water Affairs and Forestry, 2002. The development of a sanitation policy and practice in South Africa. *In*: *African Sanitation and Hygiene Conference.* Johannesburg, South Africa.
5. Doumenge, J. P. and W. H. O. P. D. Programme, 1987. *Atlas of the global distribution of schistosomiasis*, Presses Universitaires de Bordeaux Bordeaux, France.
6. El-Katsha, S. and S. Watts, 2002. *Gender, behavior, and health: Schistosomiasis transmission and control in rural Egypt*, American University in Cairo Press.
7. Engels, D., L. Chitsulo, A. Montresor and L. Savioli, 2002. The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Tropica* 82:139-146.
8. Friedman, J. F., J. D. Kurtis, S. T. McGarvey, A. L. Fraga, A. Silveira, V. Pizziolo, G. Gazzinelli, P. LoVerde and R. CorrÍa-Oliveira, 2001. Comparison of self-reported and observed water contact in an S. mansoni endemic village in Brazil. *Acta tropica* 78:251-259.
9. Gazzinelli, A., J. Bethony, L. A. Fraga, P. T. LoVerde, R. Correa Oliveira and H. Kloos,

2001. Exposure to Schistosoma mansoni infection in a rural area of Brazil. I: Water contact. *Tropical Medicine & International Health* 6:126-135.

10. Gryseels, B., K. Polman, J. Clerinx and L. Kestens, 2006. Human schistosomiasis. *The Lancet* 368:1106-1118.
11. Hopkins, O. S., D. T. Lauria and A. Kolb, 2004. Demand-based planning of rural water systems in developing countries. *Journal of water resources planning and management* 130:44-52.
12. King, C. H., 2005. Quantification of disease burden due to schistosomiasis. *In*: *Scientific Working Group on Schistosomiasis.* Geneva.
13. King, C. H., 2009. Toward the elimination of schistosomiasis. *New England Journal of Medicine* 360:106.
14. King, C. H., 2010. Parasites and poverty: the case of schistosomiasis. *Acta tropica* 113:95-104.
15. King, C. H., R. E. Blanton, E. M. Muchiri, J. H. Ouma, H. C. Kariuki, P. Mungai, P. Magak, H. Kadzo, E. Ireri and D. K. Koech, 2004. Low heritable component of risk for infection intensity and infection-associated disease in urinary schistosomiasis among Wadigo village populations in Coast Province, Kenya. *The American journal of tropical medicine and hygiene* 70:57.
16. King, C. H., R. F. Sturrock, H. C. Kariuki and J. Hamburger, 2006. Transmission control for schistosomiasis-why it matters now. *Trends in Parasitology* 22:575-582.
17. Kloetzel, K., 1992. Some personal views on the control of schistosomiasis mansoni. *Memórias do Instituto Oswaldo Cruz* 87:221-226.
18. Kloos, H., J. Ouma, H. C. Kariuki and A. E. Butterworth, 1986. Knowledge, perceptions and

health behavior pertaining to Schistosoma mansoni related illness in Machakos district, Kenya. *Tropical medicine and parasitology: official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ)* 37:171.

19. Lancet, 2004. Thinking beyond deworming. *The Lancet* 364:1993-1994.
20. Lima e Costa, M. F. F., M. H. A. Magalhães, R. S. Rocha, C. M. F. Antunes and N. Katz, 1987. Water-contact patterns and socioeconomic variables in the epidemiology of schistosomiasis mansoni in an endemic area in Brazil. *Bulletin of the World Health Organization* 65:57.
21. Mafiana, C. F., U. F. Ekpo and D. A. Ojo, 2003. Urinary schistosomiasis in preschool children in settlements around Oyan Reservoir in Ogun State, Nigeria: implications for control. *Tropical Medicine & International Health* 8:78-82.
22. Minai, M., Y. Hosaka and N. Ohta, 2003. Historical view of schistosomiasis japonica in Japan: implementation and evaluation of disease-control strategies in Yamanashi Prefecture. *Parasitology International* 52:321-326.
23. Mott, K. E., H. Dixon, E. Osei-Tutu, E. C. England, K. Ekue and A. Tekle, 1985. Indirect screening for Schistosoma haematobium infection: a comparative study in Ghana and Zambia. *Bulletin of the World Health Organization* 63:135.
24. Ndyomugyenyi, R. and J. N. Minjas, 2001. Urinary schistosomiasis in schoolchildren in Dar-es-Salaam, Tanzania, and the factors influencing its transmission. *Annals of tropical medicine and parasitology* 95:697-706.
25. Nsowah-Nuamah, N. N. N., M. E. Aryeetey, E. T. Jolayemi, Y. Wagatsuma, G. Mensah, I. K. Dontwi, F. K. Nkrumah and S. Kojima, 2004. Predicting the timing of second praziquantel treatment and its effect on reduction of egg counts in southern Ghana. *Acta*

Tropica 90:263-270.

26. Oladejo, S. O. and I. E. Ofoezie, 2006. Unabated schistosomiasis transmission in Erinle River Dam, Osun State, Nigeria: evidence of neglect of environmental effects of development projects. *Tropical Medicine & International Health* 11:843-850.
27. Opara, K. N., N. I. Udoidung and I. G. Ukpong, 2007. Genitourinary schistosomiasis among pre-primary schoolchildren in a rural community within the Cross River Basin, Nigeria. *Journal of helminthology* 81:393-397.
28. Polderman, A. M., 1984. Cost-effectiveness of different ways of controlling intestinal schistosomiasis: A case study* 1. *Social Science & Medicine* 19:1073-1080.
29. Schulz, C. R. and D. A. Okun, 1984. *Surface Water Treatment For Communities in Developing Countries*. New York, John Wiley and Sons.
30. Tucker, J. B., 1983. Schistosomiasis and water projects: breaking the link. *Environment: Science and Policy for Sustainable Development* 25:17-20.
31. Utzinger, J., R. Bergquist, P. X. Shu-Hua, B. H. Singer and P. M. Tanner, 2003. Sustainable schistosomiasis control-the way forward. *Lancet* 362:1932-1933.
32. van der Werf, M. J., S. J. de Vlas, S. Brooker, C. W. N. Looman, N. J. D. Nagelkerke, J. D. F. Habbema and D. Engels, 2003. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta tropica* 86:125-139.
33. World Health Organization, 2001. Report of the WHO informal consultation on schistosomiasis in low transmission areas: control strategies and criteria for elimination. *In*: *WHO Informal Consultation on Schistosomiasis in Low Transmission Areas.* London.
34. World Health Organization, 2006. State of the art of new vaccines: research and development. Department of Immunization, Vaccines and Biologicals, Geneva.

CHAPTER 5: EFFECTIVE CONTROL OF *SCHISTOSOMA HAEMATOBIUM* INFECTION IN A GHANAIAN COMMUNITY FOLLOWING INSTALLATION OF TRANSMISSION CONTROL INFRASTRUCTURE

Abstract

As of 2007, urogenital schistosomiasis was endemic in Adasawase, Ghana. *S. haematobium*, the causative agent of urogenital schistosomiasis, was thought to be transmitted primarily through recreational water contact. A water recreation area (WRA) was designed to serve as a primary prevention method. The WRA is a concrete pool supplied by both a borehole well and a gravity-driven rainwater collection system. The pool opened in 2009 and children were encouraged to use the facility as opposed to the local river. Annual screenings for *S. haematobium* infection in 2008, 2009, and 2010 were performed. Urine egg counts via filtration of samples through a membrane established infection incidence in the absence (2008 – 2009) and presence (2009 – 2010) of the WRA. After screening, children were treated with praziquantel by Ghana Health Services and rescreened in 2008 and 2009. In 2008, 188 of 471 (39.9%) children were egg-positive; in 2009, 70 of 345 (20.3%) of children were infected; and in 2010, reflecting incidence post-intervention, only 17 of 326 (5.2%) children were infected. The following variables were assessed via logistic regression analysis to determine correlation with infection: age, sex, distance between home and the Tini River, minutes observed at the river, low height for age, low weight for age, low Body Mass Index (BMI) for age, and previous infection status (2009 and 2010 models only). Risk factors associated with infection changed significantly during the study. Increasing age was a marginally significant risk factor for infection in 2008, along with male sex and, later, the number of minutes a child was observed to use the Tini River in 2009. In 2009, the only factors associated with infection were the number of minutes a child used the Tini River and

previous positive infection status. In 2010, only 9 children were positive for *S. haematobium* and thus, logistic regression analysis was not possible. The WRA was effective in reducing infection in Adasawase and should be tested in other water-rich areas to determine whether *S. haematobium* prevalence is substantially reduced.

Introduction

Urogenital Schistosomiasis

Schistosomiasis is a neglected tropical disease caused by parasitic trematodes of the genus *Schistosoma*. In 2006, Steinmann et al. estimated that globally, 207 million people live with schistosomiasis, but King (2010) suggests that the number is likely between 391 and 587 million people. Pathology associated with *S. haematobium* can result from chronic or acute infection. While some morbidity relates to infection intensity, other pathology is independent of worm burden and is often difficult to assess accurately, especially given long time lags between infection and the onset of severe clinical disease (King 2005). Worldwide, it is estimated that 50 to 60% of infected people *without* severe schistosomiasis exhibit symptoms (WHO 1993). Although not exclusive to schistosomiasis it is estimated that in West Africa, over 15% of the population has hematuria at any given time (van der Werf et al. 2003). As described below, there are a variety of risk factors for contracting schistosomiasis, and correspondingly, a variety of options to control morbidity and transmission. The main goal of our study was to assess *S. haematobium* infection incidence in the absence and presence of a primary prevention intervention designed to reduce water contact. We specifically evaluated a water recreation area (WRA) for its ability to reduce *S. haematobium* incidence.

Risk of Schistosoma haematobium *Infection*

Risk factors for *S. haematobium* infection tend to be location-specific; they may include age, sex, occupation, water contact practices, socioeconomic status, and distance to safe and unsafe water sources. Patterns of infection prevalence at the community scale that account for many of these variables are now being reported in the literature and may be useful for the design of interventions to reduce morbidity and parasite transmission.

Age and sex are two commonly studied risk factors for schistosome infection. The prevalence of hematuria and *S. haematobium* eggs in urine tend to increase throughout childhood and peak between the ages of 10 and 20 as a function of increasing contact with infected water (Bradley and McCullough 1973, Gryseels et al. 2006, Hammad et al. 1997, Mafiana et al. 2003, Ndyomugyenyi and Minjas 2001). The reasons for the decrease in worm burden with age are not entirely clear but may be due to changes in immunity and/or behavior (Gryseels et al. 2006). Males often have higher prevalences of infection and higher mean egg counts than do females (Ndyomugyenyi and Minjas 2001, Hammad et al. 1997), but this is not always the case (Satayathum et al. 2006, Rudge et al. 2008, Hatz et al. 1998). Sex-based differences in infection are thought to result from different patterns of behavior, and not from an underlying genetic predisposition to infection.

Previous infection with schistosomes is a complex risk factor and may predict likelihood of current infection. Previous infection may indicate the presence of behavior that increases the subsequent risk of reinfection, but could also be associated with a relatively high likelihood of recent treatment with praziquantel, the drug of choice to kill schistosomes (Hammad et al. 1997). Finally, previous infection may correlate with acquired immunity.

Water contact behavior has been examined by a number of researchers with varying results. Clothes washing, water collection, swimming/bathing, and fishing have all been identified as risk factors for schistosome infection (Hammad et al. 1997, Handzel et al. 2003). Rudge et al. (2008) believe that the lack of a general consensus about risk factors may be partly due to an "inadequate understanding of how water contact translates into exposure, or indeed how exposure translates into infection intensity." For example, Hammad et al. (1997), Handzel et al. (2003), and Stothard et al. (2009) have found correlations between water contact and infection with schistosomes, but Satayathum et al. (2006) working in Kenya and Pereira et al. (2010) working in Brazil did not. Bethony et al. (2001) note that the "the paradigm of exposure to potentially infective water and number of eggs excreted [may be] too simple." Simple exposure to potentially infective water and infection status may not show a strong correlation. However, studies have shown that proximity to surface water contaminated with schistosomes and/or snails is significantly associated with infection prevalence in Kenya (Handzel et al. 2003) and Tanzania (Rudge et al. 2008).

Control of Urogenital Schistosomiasis

In 1993, the World Health Organization (WHO) stated that control of schistosomiasis should be accomplished within the context of the existing primary health care system, and that a long-term commitment (10 to 20 years) to schistosomiasis control is necessary (WHO 1993). As of 2011, there is very little effective schistosomiasis control in sub-Saharan Africa. Many of the control efforts that began in the 1980s were funded by external donors, and were discontinued when external financial support was discontinued (Engels et al. 2002). In endemic countries with relatively small health-related budgets, it is also necessary to consider schistosomiasis control in

the context of controlling other, more pressing public health problems. Control programs can be broadly categorized into transmission control and/or morbidity control initiatives. An appropriate control strategy depends on factors such as local conditions, infection risk factors, available financial resources, and community preferences.

Control options for schistosomiasis were recently reviewed by King (2010); the major control options currently in use include: mass drug administration (MDA); water, sanitation and hygiene programs; education and behavior change programs; and occasionally, snail control. There is no single solution that is appropriate for every setting. For example, Stothard et al. (2009) argue for the need to address *S. haematobium* transmission via improved access to clean water, education, and behavior change. Satayathum et al. (2006) determined that annual treatment of egg-positive school-aged children in Kenya could not reduce infection prevalence below 14% between 1984 and 1992, which suggests that additional strategies, such as the provision of safe water, should supplement MDA. Intensive health education in Senegal was evaluated by Sow et al. (2003); after seven years duration, knowledge of *S. mansoni* infection, transmission, symptoms, and treatment remained very low among both children and adults. The authors concluded that (a) community-driven control would be more effective than a vertical approach implemented by outsiders and (b) behavior change may not occur when individuals lack access to infrastructure that allows them to minimize their risk of infection with schistosomes (Sow et al. 2003). The literature demonstrates that MDA continues to be necessary, but in some settings, it must be coupled with water, sanitation, and hygiene-based infrastructure.

Analysis of Risk Factors via Logistic Regression

Logistic regression (LR) can be used for prediction (Bagley et al. 2001, Bewick et al. 2005), hypothesis testing, or the determination of the statistical significance of covariates (Bagley et al. 2001, Hammad et al. 1997). Potential risk factors for developing urogenital schistosomiasis were evaluated via LR analysis by Clennon et al. (2006), Nsowah-Nuamah et al. (2001), and Hammad et al. (1997). Nsowah-Nuamah et al. (2001) developed a logistic regression model to characterize infection among individuals living in the presence or absence of interventions such as well and latrine construction and weed removal from surface water bodies. Two years post-intervention, people living in intervention areas had 0.15 times the odds of infection as compared with people living elsewhere. Clennon et al. (2006) found via LR analysis that *S. haematobium* infections were clustered within about 1,500 meters of water bodies containing *Bulinus* snails. Hammad et al. (1997) found that individuals who self-reported recreational contact (swimming or bathing) with contaminated water were 1.5 times a likely as their non-recreating peers to be infected with *S. haematobium*, but the overall conclusion of the authors was that age-acquired immunity may strongly influence risk of infection.

Spatial Risk Factors and Geographic Information Systems (GIS)

In schistosomiasis modeling, GIS and remote sensing can be used to assess risk based on a number of factors including air temperature, rainfall, vegetation type and density, and population density (Bavia et al. 2001). Because infections are heterogeneously distributed (Brooker et al. 2002), this technique has limited applicability to community-specific, small-scale studies. Based on the results of predictive modeling, GIS may be used by decision makers to decide where and when to implement control strategies (Brooker et al. 2002). In addition to predictive modeling,

spatial data related to patterns of *S. haematobium* infection, water contact behavior, and environmental factors can be collected at an appropriately small scale within communities and the statistical significance of spatial data can be assessed.

Risk factors for infection vary among and within communities. Additional studies are needed to document the spatial heterogeneity of schistosome infection and to better characterize significant risk factors other than climate and terrain (Brooker 2007, Raso et al. 2005). For example, Clennon et al. (2004) mapped home and water contact locations through the use of very high resolution (1 to 4 m^2) remotely-sensed imagery in a community in Kenya with 306 households and concluded that children under 6 who live near water contact sites may have more exposure to contaminated water and thus may develop immunity to reinfection earlier than children who live farther away.

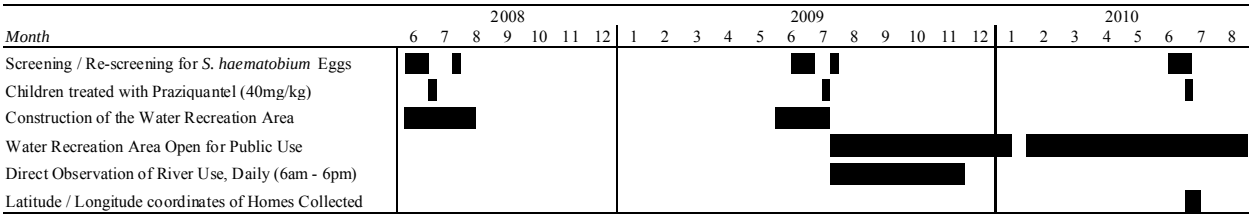
Materials and Methods

This research focused on *S. haematobium* infections in children residing in Adasawase, Ghana. The infection is transmitted via recreational contact with the Tini River, the only source of contact with surface water. We hypothesized that the provision of a WRA would reduce *S. haematobium* infection rates. This hypothesis was tested by collaborating with the community to design and construct a WRA, quantifying infection incidence before and after the structure was opened, and identifying and controlling for confounding factors that affect infection rates. Each task is described in detail below.

Study Design

The study was carried out in the town of Adasawase, Ghana. The location was selected based on a relatively high prevalence of *S. haematobium* infection as reported by the Chief of Adasawase in December 2007. The study was conducted over the course of three years (Figure 1). In 2008, infection prevalence for *S. haematobium* in the community was quantified, all children were treated with praziquantel by Ghana Health Services (WHO 2002), and construction of the WRA began. Our protocol called for multiple screening tests to improve the accuracy of true prevalence and incidence estimates (Kosinski et al. 2011). In 2009, reinfection in the community was quantified in the absence of the WRA, children were again treated with praziquantel, and the WRA was opened for public use. Directly after the WRA was opened for public use, water contact at the local river was observed daily for four months by a trained member of the community. In 2010, reinfection was quantified after the WRA was used for one year. Egg-positive children were specifically treated with praziquantel, but any child who wished to take praziquantel was treated. The WHO (2002) recommends selective treatment when less than 10% of children aged 6 to 15 years is positive for *S. haematobium* via parasitological diagnosis (screening method not specified).

Figure 1: Main study activities carried out in Adasawase, Ghana (2008 – 2010).



Study Population

Adasawase has a population of approximately 2,000 residents. *S. haematobium* infection rates were monitored in school-age children in the town. Children invited to participate in the study were 8 years of age and older, residents of Adasawase, and enrolled in one of three schools in Adasawase as of June 2008, June 2009, and/or June 2010. The schools of Adasawase include one junior high school and two primary schools. The number, percentage and age of children who were screened at least 3 times in any given year are shown in Table 1. Not all children screened 3

Table 1: The number, percentage, and ages of children screened at least 3 times in any given year: Adasawase, Ghana 2008 – 2010.

	2008 - GIRLS			2009 - GIRLS			2010 - GIRLS		
Birth Year	Screened 3+ Times	Total Number Enrolled	(%) Screened 3+ Times	Screened 3+ Times	Total Number Enrolled	(%) Screened 3+ Times	Screened 3+ Times	Total Number Enrolled	(%) Screened 3+ Times
2002							21 /	21	100.0
2001				14 /	21	66.7	20 /	23	87.0
2000	0 /	10	0.0	18 /	24	75.0	14 /	19	73.7
1999	16 /	37	43.2	29 /	34	85.3	24 /	26	92.3
1998	10 /	22	45.5	15 /	21	71.4	17 /	20	85.0
1997	24 /	35	68.6	27 /	30	90.0	22 /	25	88.0
1996	15 /	24	62.5	13 /	18	72.2	13 /	15	86.7
1995	14 /	19	73.7	10 /	15	66.7	7 /	7	100.0
1994	9 /	13	69.2	8 /	13	61.5	8 /	9	88.9
1993	7 /	8	87.5	7 /	8	87.5	3 /	6	50.0
1992	17 /	24	70.8	6 /	8	75.0	4 /	5	80.0
1991	5 /	9	55.6	1 /	2	50.0			
1990	2 /	2	100.0						
Total	119 /	203	58.6	148 /	194	76.3	153 /	176	86.9

	2008 - BOYS			2009 - BOYS			2010 - BOYS		
Birth Year	Screened 3+ Times	Total Number Enrolled	(%) Screened 3+ Times	Screened 3+ Times	Total Number Enrolled	(%) Screened 3+ Times	Screened 3+ Times	Total Number Enrolled	(%) Screened 3+ Times
2002							15 /	18	83.3
2001				10 /	19	52.6	27 /	33	81.8
2000	0 /	9	0.0	15 /	19	78.9	20 /	22	90.9
1999	18 /	42	42.9	32 /	39	82.1	32 /	34	94.1
1998	16 /	34	47.1	22 /	32	68.8	23 /	27	85.2
1997	9 /	18	50.0	15 /	19	78.9	17 /	19	89.5
1996	18 /	26	69.2	13 /	22	59.1	15 /	19	78.9
1995	31 /	48	64.6	32 /	44	72.7	34 /	39	87.2
1994	16 /	18	88.9	10 /	16	62.5	9 /	14	64.3
1993	4 /	18	22.2	7 /	14	50.0	2 /	2	100.0
1992	10 /	31	32.3	7 /	13	53.8	10 /	12	83.3
1991	8 /	19	42.1	2 /	3	66.7	3 /	3	100.0
1990	3 /	3	100.0	1 /	1	100.0			
1989	1 /	2	50.0						
1988	0 /	1	0.0	0 /	1	0.0	0 /	1	0.0
Total	134 /	269	49.8	166 /	242	68.6	207 /	242	85.5

or more times in any given year were previously treated with praziquantel and re-screened; thus, the number of students whose data was used in 2009 and 2010 is smaller than the number screened 3 or more times.

Consent: Institutional Review Boards

This study was approved by the Social, Behavioral, and Educational Institutional Review Board (IRB) of Tufts University and the IRB of the Noguchi Memorial Institute for Medical Research (NMIMR). Each child who participated provided verbal assent. The Chief of Adasawase and the head of each school gave written permission to the study team to conduct the research; these individuals communicated with parents and community members about the nature of the study.

Water Recreation Area

Children who become infected with *S. haematobium* in Adasawase were most likely to do so via contact with the Tini River, the only local surface water body. A WRA was selected as an intervention option by the community and designed and constructed to reduce contact of local school children with this river. The design, construction, operation, and maintenance of the WRA are described in detail elsewhere (Kosinski et al., under revision, Journal of Water, Sanitation and Hygiene for Development; also see Chapter 4 of this thesis). The WRA was opened for public use in July, 2009.

Parasitological Data

S. haematobium infection prevalence was quantified in June of 2008 and incidence in June/July of 2009 and 2010. Infection status was determined by urine filtration. Urine was collected in conical 50 mL tubes between 10:00 am and 2:00 pm from children who were present at school

on designated screening days. Each urine sample was tested for eggs via filtration, as described below. Each sample was also tested for hematuria, but these data are not reported here.

In 2008, schools were visited up to 7 times to request urine samples on 3 different days for a total of 3 samples per child (1 sample per day). In 2009 and 2010, schools were visited up to 9 times to request urine samples from each child on 4 different days for a total of 4 samples per child (1 sample per day). Once a child provided 3 (2008) or 4 (2009 and 2010) samples, (s)he was not asked for additional samples. Data from children who provided at least 3 samples are presented here. A flow chart of the study design is available upon request from the primary author (see Appendix A).

Urine samples were tested for *S. haematobium* eggs via filtration through Nucleopore membranes (25 mm diameter, 12.0 μm pore size; Sterlitech Corporation, Kent, Washington). Urine was drawn into a 10 mL syringe and then discharged through a new Nucleopore membrane. Filtered urine was discarded. Membranes were removed from filter holders with forceps and placed egg-side-down on glass slides and examined under 10x magnification. For every sample, all *S. haematobium* eggs on each membrane were counted by the same trained and experienced laboratory technician from the NMIMR.

Filtration of a single 10 mL sub-sample of urine is a common practice (van der Werf et al. 2003). In this study, samples were tested either by extracting a 10 mL sub-sample of urine and filtering it, or by filtering the entire urine volume. The objective of this study was to accurately identify 'true positive' individuals while collecting no more than four samples per participant, at the request of school heads. Filtration of the full volume of a urine sample is a slightly more sensitive method than filtration of a 10 mL sub-sample. For comparison with other studies, the first urine sample that each child submitted was tested by filtering a 10 mL sub-sample. The

majority of subsequent samples were tested as 10 mL sub-samples. When full volume filtration was performed in 2008, 4 children in the study presented with less than 0.5 eggs/10 mL urine and in 2009, 3 children presented with a maximum of less than 0.5 eggs/10 mL urine. Filtration results were recorded as total eggs counted and converted to eggs per 10 mL urine. For logistic regression analysis, data were reduced to a binary score of positive or negative for *S. haematobium* eggs; thus, actual egg counts were not utilized here.

Directly Observed Behavioral Data

Water contact may be assessed through a variety of methods including self-reporting, direct observation, or a combination of these. In this research, direct observation was used to record behavior data. A single local community member was trained in the collection of directly observed behavioral data at the Tini River, the only recreational water contact site used by the community. The trained individual is a resident of Adasawase and had established rapport with children enrolled in the study, and well as with other community members such as teachers and parents. He was instructed to answer questions about the study if asked, and not to record information about anyone who did not wish to be observed (no one made this request).

This individual observed the river from 6:00 am to 6:00 pm for 14 days between July 5 and July 31, 2009. He observed the river from 6:00 am to 6:00 pm 7 days per week (84 hours/week) from August to November 2009. The observer was compensated for his time. The following data were collected for each school-aged person who visited the river: name, age, school attended, school class, time of day, minutes in contact with river water, and activities performed (swimming, washing/bathing, domestic water collection, and washing of clothing or utensils in the river).

Anthropometric Data

Anthropometric data were collected from each child with respect to the child's height in centimeters and weight in kilograms. Body mass index (BMI) was calculated based on the child's height and weight according to the formula: [weight (kg) / height (m^2)]. For height measurements, children stood erect and barefoot against a stadiometer. In 2008 and 2009, height was recorded to the nearest 0.10 cm; in 2010, height was recorded to the nearest 0.50 cm, rounding up. For weight measurements, a digital scale was used to record the child's weight to the nearest 0.1 kg in 2008 and 2009. In 2010, weight was recorded with a mechanical scale to the nearest 0.1 kg. Children wore school uniforms when they were weighed.

A child's status as having low height for age, low weight for age, and/or low BMI for age was determined based on the "Simplified Field Tables" from the WHO (2007). The birth days and birth months of children were unknown; the children were not able to self-report their birth day or birth month. The birth year of each child was obtained from school records. The cutoff point for low height for age was -2 standard deviations (SD) for the child's age in years plus 6 months, inclusive. The cutoff point for low weight for age was the fifth percentile for the child's age in years plus 6 months, inclusive. The cutoff point for low BMI for age was the third percentile for the child's age in years plus 6 months, inclusive.

Residential and Spatial Data

In July 2010, two members of the research team created a spatial plan of Adasawase to determine the locations of homes of children enrolled in the study. One team member is from Adasawase and was known by community members to be collaborating with the study team with regard to schistosomiasis control. This team member located all houses, explained the study to

participants, obtained verbal consent to participate in the study, and asked questions about school-aged individuals in each home. The other team member used a handheld global positioning system (GPS) unit (Garmin GPS 60 Portable Navigator, Garmin, Ltd.) to collect the latitude and longitude coordinates of homes and well-known landmarks in Adasawase. He recorded data in a field notebook. Household members were verbally asked to provide the name, age, sex, grade level, and school of each school-aged child in the household. These data were recorded in a notebook and then manually matched to data corresponding to the child's infection status and previous demographic data. When an exact match of information was not found, the relevant household was revisited and follow-up questions were asked to rectify any discrepancies. For example, minor misspellings of names were acceptable, but discrepancies of more than 2 years in a child's reported age or report that a child attended a school other than the one on record necessitated revisiting a home for additional information.

GIS layers were constructed by digitizing satellite imagery against the latitude and longitude coordinates of landmarks collected with the handheld GPS unit. Once the satellite image was georectified (World Geodetic System 1984, 30N), walking paths, roads, surface water, and points of interests were manually digitized from the image. House locations were imported into ArcGIS (version 9.3.1) from the handheld GPS unit. The objective was to determine the locations of houses with respect to the Tini River.

Data Entry

Data collected in this research included parasitological data (hematuria and egg data), behavioral data, anthropometric data, and spatial data. All data were entered into SPSS 14.0 (SPSS Inc.,

Chicago, IL). All data were double-entered. Logistic regression was performed on the data as described below.

Logistic Regression

Variables were tested via LR analysis (enter method) to determine whether or not their association with infection status is statistically significant. For 2008 infection status, variables tested include: age (ordinal), sex (nominal), the distance between a child's home and the Tini River (ordinal), and the number of minutes a child was observed to be in contact with the river in 2009 (continuous). These same risk factors were considered for 2009, in addition to the variable 'previous infection status' (nominal). Note that observation of behavior in 2009 took place after children were screened in 2008 and 2009; the variable is used as a proxy for behavior that occurred prior to 2008 and 2009 screening. A final model for each LR analysis was chosen once all variables in the model were either statistically significant or biologically plausible *and* near the attainment of statistical significance ($p < \approx 0.075$).

Results and Discussion

The main objective of this study was to test the hypothesis that the incidence of *S. haematobium* infection among a population of schoolchildren would decrease in the presence of a WRA. We tested this hypothesis by assessing incidence of *S. haematobium* infection before and after WRA construction. For data analysis, study participants were separated into five cohorts based on age, school enrollment, screening status, treatment status, and whether previous infection status was known (P.I.S.K.) (Table 2). The 2008 cohort (2008) was chosen based on age, school enrollment,

and participation in three screenings. The 2009 and 2010 cohorts were both chosen based on: age; school enrollment; participation in three or more screenings in the relevant year (2009 or 2010); treatment with praziquantel in the previous year; and negative *S. haematobium* status in the previous year at baseline. The 2009-P.I.S.K. and 2010-P.I.S.K. cohorts were both chosen according to these same criteria, in addition to the criteria that children were screened three or more times in the previous year and their previous infection status was known (P.I.S.K.).

Table 2: Criteria by which study participants in Adasawase, Ghana were included in specific cohorts based on age, school enrollment, screening status, and treatment status.

	Criteria for Inclusion in Data Analysis				
	2008	2009	2009-P.I.S.K.	2010	2010-P.I.S.K.
8 years of age or older	X	X	X	X	X
Enrolled in school in June of screening year	X	X	X	X	X
Screened 3+ times in June/July of screening year	X	X	X	X	X
Screened 3+ times in June/July of previous year			X		X
Treated with praziquantel in previous year		X	X	X	X
Screened 1 time after previous praziquantel treatment and found negative **and/or** tested negative on 3+ screenings prior to praziquantel in previous year		X	X	X	X
Total number of participants	254	220	137	246	186

Prevalence and Incidence of Infection Pre- and Post-Intervention

The baseline prevalence of *S. haematobium* infection in 2008 and the incidence of infection in 2009 and 2010 are shown in Table 3. In 2008, data from all children in the cohort "2008" (see Table 2) were used to calculate cross-sectional prevalence. The objective was to determine the cross-sectional prevalence of *S. haematobium* infection among study participants, and not necessarily among the entire population of school-aged children in Adasawase. The prevalence of infection among children who were screened 3 times may not be comparable to the prevalence among children who were screened fewer times or not screened at all. Note that this criterion of

participating in at least 3 screenings was applied in each year of the study and thus, infection in 2008, 2009, and 2010 represents a comparison among similar populations.

Central Finding

In 2008, 36.1% (n = 43/119) of girls and 50.7% (n = 69/134) of boys (cohort = "2008") were positive for *S. haematobium* eggs. In 2009, 13.3% (n = 13/98) of girls and 23.8% (n = 29/122) of boys were infected with *S. haematobium* (cohort = "2009"), reflecting annual incidence in the absence of the WRA. In 2010, only 1.9% (2/105) of girls and 5.0% (7/141) of boys were positive for *S. haematobium* eggs (cohort = "2010"), reflecting annual incidence in the presence of the WRA.

Table 3: Prevalence (2008) and incidence of *S. haematobium* infection (2009 and 2010), as determined by *S. haematobium* egg count, stratified by age and sex. Cross-sectional prevalence in 2008 is based on data from the 2008 cohort. Incidence in 2009 and 2010 is based on data from cohorts 2009 and 2010, respectively.

	2008 - GIRLS			2009 - GIRLS			2010 - GIRLS		
Birth Year	*S. haematobium* Egg Positive	Screened 3+ Times	(%)	*S. haematobium* Egg Positive	Screened 3+ Times	(%)	*S. haematobium* Egg Positive	Screened 3+ Times	(%)
2002							0 /	6	0.0
2001				1 /	1	100.0	0 /	16	0.0
2000				1 /	7	14.3	1 /	9	11.1
1999	2 /	16	12.5	2 /	23	8.7	0 /	20	0.0
1998	1 /	10	10.0	1 /	10	10.0	0 /	11	0.0
1997	10 /	24	41.7	4 /	19	21.1	1 /	15	6.7
1996	7 /	15	46.7	0 /	11	0.0	0 /	7	0.0
1995	5 /	14	35.7	1 /	10	10.0	0 /	7	0.0
1994	3 /	9	33.3	1 /	6	16.7	0 /	8	0.0
1993	3 /	7	42.9	1 /	5	20.0	0 /	3	0.0
1992	9 /	17	52.9	1 /	5	20.0	0 /	3	0.0
1991	2 /	5	40.0	0 /	1	0.0			
1990	1 /	2	50.0						
Total	43 /	119	36.1	13 /	98	13.3	2 /	105	1.9

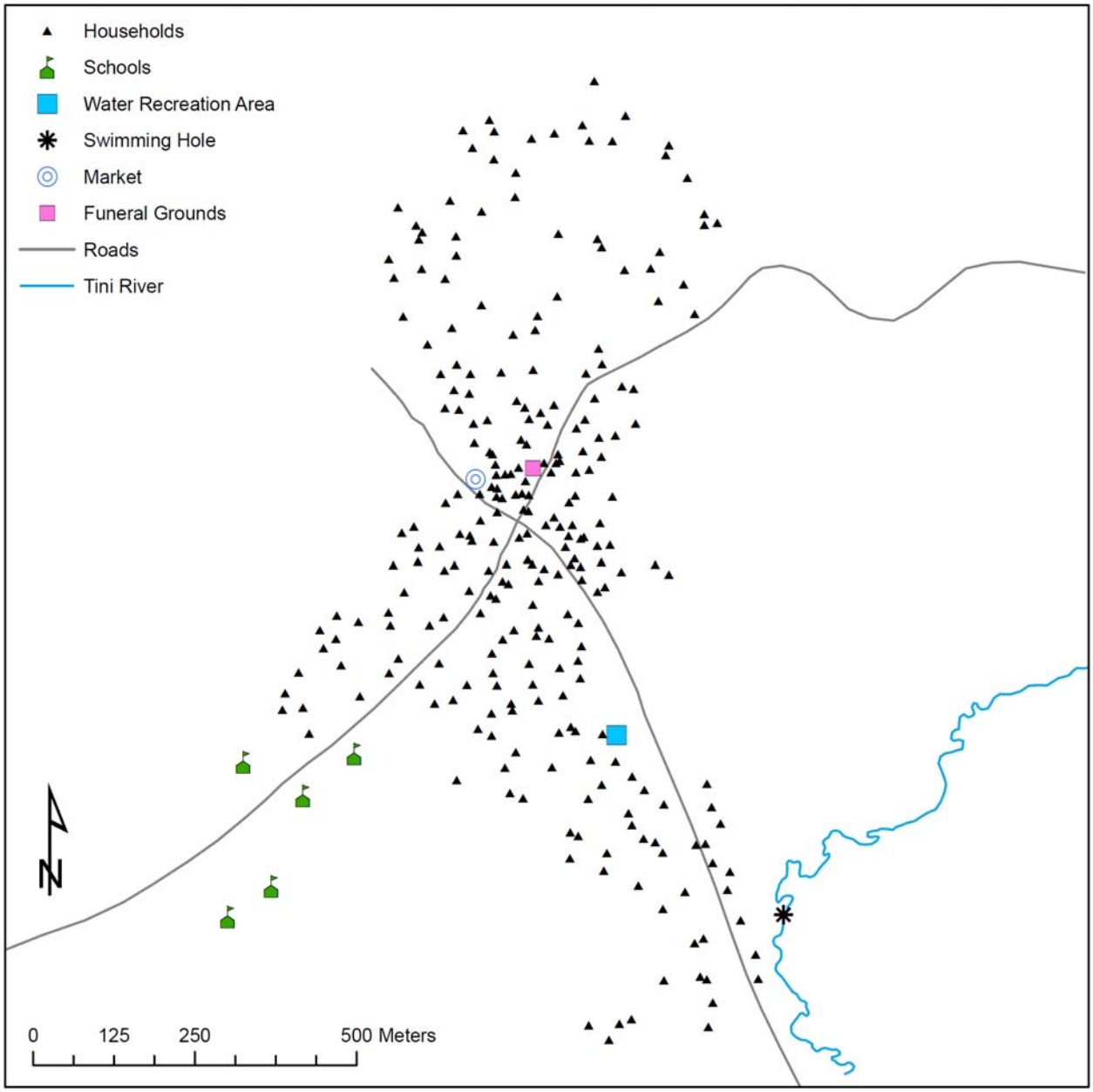
	2008 - BOYS			2009 - BOYS			2010 - BOYS		
Birth Year	*S. haematobium* Egg Positive	Screened 3+ Times	(%)	*S. haematobium* Egg Positive	Screened 3+ Times	(%)	*S. haematobium* Egg Positive	Screened 3+ Times	(%)
2002							0 /	2	0.0
2001				0 /	1	0.0	0 /	16	0.0
2000				1 /	6	16.7	0 /	12	0.0
1999	10 /	18	55.6	6 /	28	21.4	2 /	26	7.7
1998	6 /	16	37.5	4 /	19	21.1	2 /	20	10.0
1997	4 /	9	44.4	3 /	11	27.3	0 /	14	0.0
1996	11 /	18	61.1	5 /	11	45.5	1 /	13	7.7
1995	16 /	31	51.6	7 /	24	29.2	2 /	22	9.1
1994	9 /	16	56.3	2 /	9	22.2	0 /	7	0.0
1993	2 /	4	50.0	1 /	5	20.0	0 /	2	0.0
1992	4 /	10	40.0	0 /	7	0.0	0 /	5	0.0
1991	4 /	8	50.0				0 /	2	0.0
1990	2 /	3	66.7	0 /	1	0.0			
1989	0 /	1	0.0						
Total	68 /	134	50.7	29 /	122	23.8	7 /	141	5.0

Spatial Plan of Adasawase

A spatial plan of Adasawase was created using GIS data collected in 2010 (Figure 2). The home locations for 71 out of 254 children in the 2008 cohort were available in 2010. The home locations for 86 out of 220 children were available for the 2009 cohort, and for 117 out of 246 children in the 2010 cohort. The home locations for the rest of the children in each cohort were not able to be determined.

In the analysis of the spatial data, several simplifying assumptions were made. As a measure of distance, we employed the simple linear distance between homes and the Tini River, in contrast to calculating actual walking distance. In addition, we assumed that children reported to live at a particular home in 2010 resided in that same home in 2008 and 2009 if they were enrolled in school. The distance between a child's home and the Tini River was calculated in meters; the data were then reduced to an ordinal number corresponding to 1 = "close" (< 500 m), 2 = "medium distance" (≥ 500 m and ≤ 1,000 m), and 3 = "far" (> 1,000 m). These cutoff points were assigned by the research team based on the scale of the community.

Figure 2: Spatial plan of Adasawase, Ghana; spatial data (latitude and longitude coordinates) were collected in 2010 with a handheld GPS unit (Garmin GPS 60 Portable Navigator, Garmin, Ltd.).



Directly Observed Behavior at the Tini River

The Tini River was observed by one trained individual for a total of 97,920 minutes between July and November of 2009. The individual observed the river for 14 days in July 2009, and every day between 1 August and 30 November 2009 from approximately 6:00am to 6:00pm. It

was generally accepted in the community that use of the river after 6:00pm was very rare. Children who were egg-positive for *S. haematobium* in any given year were more likely than their sex-matched peers to (a) use the river and (b) use the river for longer periods (Table 4). Directly observed behavior in 2009 was considered a proxy for behavior in 2008, 2009, and 2010; it was considered in all regression models and found to be a highly significant predictor of infection status in all models (see Tables 6a, 6b, and 6c).

Table 4: Directly observed use of the Tini River by girl and boy children between July and November 2009, Adasawase, Ghana; only data from children who were observed to use the river at least once during this time period are presented.

	Use of the Tini River - GIRLS					
	2008		2009-P.I.S.U.		2010-P.I.S.U.	
	S. haematobium-positive	*S. haematobium*-negative	*S. haematobium*-positive	*S. haematobium*-negative	*S. haematobium*-positive	*S. haematobium*-negative
Total number of children	43	76	13	85	2	103
Number of children observed using the Tini River in 2009	8	4	5	8	1	13
Percentage of total children using the Tini River in 2009	18.6% (8/43)	5.3% (4/76)	38.5% (5/13)	9.4% (8/85)	50.0% (1/2)	12.6% (13/103)
Total time the Tini River was used in 2009 (min)	2245	900	2140	1455	475	3765
Total contact time per person, Range (min)	20 to 685	20 to 475	20 to 685	20 to 475	475	20 to 685
Mean contact time per person who used the Tini River (min ± s.d.)	280 ± 254	225 ± 219	428 ± 268	177 ± 157	475	288 ± 240
Range of number of contacts per person who used the Tini River (contacts)	2 to 41	1 to 32	2 to 41	1 to 36	32	1 to 41
Mean nummber of minutes per peson observed at the Tini River - Morning	128	109	177	86	225	138
Mean nummber of minutes per peson observed at the Tini River - Afternoon	131	108	211	82	215	128
Mean nummber of minutes per peson observed at the Tini River - Evening	22	9	40	11	35	23

	Use of the Tini River - BOYS					
	2008		2009-P.I.S.U.		2010-P.I.S.U.	
	S. haematobium-positive	*S. haematobium*-negative	*S. haematobium*-positive	*S. haematobium*-negative	*S. haematobium*-positive	*S. haematobium*-negative
Total number of children	69	66	29	93	7	134
Number of children observed using the Tini River in 2009	12	7	11	11	1	33
Percentage of total children using the Tini River in 2009	17.4% (12/69)	10.6% (7/66)	37.9% (11/29)	11.8% (11/93)	14.3% (1/7)	24.6% (33/134)
Total time the Tini River was used in 2009 (min)	3889	870	3821	1550	330	6361
Total contact time per person, Range (min)	5 to 832	10 to 345	95 to 832	5 to 585	330	5 to 832
Mean contact time per person who used the Tini River (min ± s.d.)	324 ± 252	124 ± 128	348 ± 236	141 ± 171	330	191 ± 199
Range of number of contacts per person who used the Tini River (contacts)	1 to 53	1 to 19	5 to 53	1 to 52	23	1 to 53
Mean nummber of minutes per peson observed at the Tini River - Morning	155	39	177	62	180	90
Mean nummber of minutes per peson observed at the Tini River - Afternoon	152	83	157	68	105	96
Mean nummber of minutes per peson observed at the Tini River - Evening	17	2	14	10	45	7

Risk Factors of Infection

Univariable Analysis of Risk Factors

Three separate univariable analyses of potential risk factors for infection with *S. haematobium* were conducted to identify risk factors that were likely to be significant in multivariable models. The dichotomous outcome variable in each case is *S. haematobium* infection status in 2008, 2009, and 2010. Results of univariable analysis are shown in Table 5. After univariable analysis, potential predictor variables with respect to multivariable logistic regression were identified based on whether or not they were significant ($p < 0.05$) or marginally significant ($p < 0.10$). Surprisingly, in none of the univariable analyses was "age" significantly associated with infection. Prior to the intervention (2009 model only), variables that were associated with infection include: sex, the distance between a child's home and the Tini River, the number of minutes observed using the river, and previous infection status. Post-intervention in 2010, none of the variables remained significant as predictors of infection status.

Table 5: Univariable analysis of risk factors for infection with *S. haematobium*; note that direct observation of behavior at the Tini River occurred between July and November of 2009; observed behavior is considered in models of 2008, 2009, and 2010 infection status. The outcome variable in each case is *S. haematobium* infection status (by egg count) for the corresponding year (2008, 2009, 2010).

	2008				
				95% C.I. for Exp(B)	
Variable	**n**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Age	254	0.133	1.08	0.977	1.193
Sex (Female)	254	0.017	1.848	1.117	3.058
Distance between Home and the Tini River	71	0.058	0.419	0.170	1.030
Minutes Observed at River (Jul - Nov 2009)	254	0.008	1.004	1.001	1.007
Low Height for Age	252	0.799	1.067	0.648	1.755
Low Weight for Age	252	0.688	0.900	0.539	1.504
Low BMI for Age	252	0.583	1.189	0.641	2.202

	2009				
				95% C.I. for Exp(B)	
Variable	**n**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Age	220	0.920	0.993	0.859	1.147
Sex (Female)	220	0.052	2.039	0.995	4.177
Distance between Home and the Tini River	86	0.020	0.321	0.124	0.833
Minutes Observed at River (Jul - Nov 2009)	220	0.000	1.006	1.003	1.009
Low Height for Age (Normal)	196	0.439	1.329	0.646	2.733
Low Weight for Age (Normal)	220	0.218	1.627	0.750	3.532
Low BMI for Age (Normal)	196	0.636	1.251	0.495	3.160
Previous Infection Status (Negative)	137	0.000	8.010	2.812	22.814

	2010				
				95% C.I. for Exp(B)	
Variable	**n**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Age	246	0.811	1.032	0.800	1.331
Sex (Female)	246	0.223	2.690	0.547	13.223
Distance between Home and the Tini River	117	0.260	2.456	0.514	11.730
Minutes Observed at River (Jul - Nov 2009)	248	0.242	1.002	0.999	1.005
Low Height for Age (Normal)	246	0.065	4.485	0.912	22.049
Low Weight for Age (Normal)	237	0.751	1.257	0.307	5.156
Low BMI for Age (Normal)	235	0.594	0.564	0.069	4.635
Previous Infection Status (Negative)	186	0.265	2.266	0.538	9.541

Baseline S. haematobium *Infection*

A logistic regression model was developed for the dichotomous outcome variable *S. haematobium* infection status in 2008. Variables considered in the model include: age, sex, distance between home and the Tini River, minutes observed at the river, low height for age, low

weight for age, and low Body Mass Index (BMI) for age. Sex and minutes observed at the river were significant in the model (Table 6a); age is of marginal significance but is retained in the model for comparison with other studies. The distance between a child's home and the Tini River may have been a relevant factor for infection, but the sample size for this variable was relatively small (n = 71) and it was not significant in the final model. The model correctly identified 81.0% of negative children and 32.4% of positive children, indicating that while relevant, not all variability in infection status is captured by these explanatory factors. The final model was chosen based on the significance of each explanatory variable and on biological plausibility (Hosmer and Lemeshow = 0.567). The model shows that increasing age was marginally significant (p = 0.070); males were more likely than females to be infected (Exp(B) = 1.775, p = 0.030); and for every additional minute spent contacting the Tini River, risk of infection increases by 1.004 (p = 0.006).

Table 6a: Logistic regression model including variables associated with *S. haematobium* infection in 2008, as assessed by egg count; reference conditions shown in parentheses.

								95.0% C.I.for Exp(B)	
Variable	**n**	**B**	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Age	254	0.095	0.053	3.278	1	0.070	1.100	0.992	1.219
Sex (Female)	254	0.574	0.264	4.724	1	0.030	1.775	1.058	2.979
Minutes Observed at River (Jul - Nov 2009)	254	0.004	0.002	7.553	1	0.006	1.004	1.001	1.007
Constant		-1.884	0.702	7.212	1	0.007	0.152		

Pre-Intervention S. haematobium *Infection*

Two different logistic regression models were developed for the dichotomous outcome variable "*S. haematobium* infection status in 2009" (Table 6b and 6c). In the first model (Table 6b), variables considered include: age, sex, distance between home and the Tini River, minutes observed at the river, low height for age, low weight for age, low BMI for age, and 2008 infection status. To assess the significance of the variable '2008 infection status', only data from

children in the group "2009-P.I.S.K." (Table 2) were considered (n = 137); to ensure accuracy, these children were screened at least three times for schistosome eggs in 2008. The number of minutes observed at the river (Exp(B) = 1.005, p = 0.004) and 2008 infection status (Exp(B) = 6.210, p = 0.001) were significantly associated with *S. haematobium* infection in 2009, but none of the other variables were significant (Table 6b). The model correctly identified 98.2% of negative children and 25.9% of positive children (Hosmer and Lemeshow = 0.596). The final model was chosen based on the significance of each explanatory variable and on biological plausibility.

Table 6b: Logistic regression model including variables associated with *S. haematobium* infection in 2009, as assessed by egg count; reference conditions shown in parentheses.

								95.0% C.I.for Exp(B)	
Variable	**n**	**B**	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Minutes Observed at River (Jul - Nov 2009)	137	0.005	0.002	8.195	1	0.004	1.005	1.002	1.009
2008 Infection Status (Negative)	137	1.826	0.554	10.862	1	0.001	6.210	2.096	18.396
Constant		-2.828	0.480	34.706	1	0.000	0.059		

A second logistic regression model was developed for the dichotomous outcome variable "*S. haematobium* infection status in 2009" that did not consider 2008 infection status as an explanatory variable (Table 6c). In the second model, variables considered include: age, sex, distance between home and the Tini River, minutes observed at the river, low height for age, low weight for age, and low BMI for age. The number of minutes observed at the river was significantly associated with infection in 2009 (Exp(B) = 1.006, p < 0.000), and male sex was marginal significant as a risk factor (Exp(B) = 2.184, p = 0.051). The model correctly identified 97.8% of negative children and 21.4% of positive children (Hosmer and Lemeshow = 0.704). The final model was chosen based on the significance of each explanatory variable and on biological plausibility; variables that are not included were not significant.

Table 6c: Logistic regression model including variables associated with *S. haematobium* infection in 2009, as assessed by egg count; the variable '2008 Infection Status' was not consider as a potential explanatory variable. Reference conditions are shown in parentheses.

								95.0% C.I.for Exp(B)	
Variable	**n**	**B**	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Minutes Observed at River (Jul - Nov 2009)	220	0.006	0.001	17.740	1	0.000	1.006	1.003	1.009
Sex (Female)	220	0.781	0.401	3.799	1	0.051	2.184	0.996	4.792
Constant		-2.266	0.342	43.936	1	0.000	0.104		

Post-Intervention S. haematobium *Infection*

Two girls and 7 boys were infected with *S. haematobium* following construction of the WRA. Because of the small number of infected children, logistic regression analysis was not possible. Instead, the potential risk factors associated with infection are presented for each child (Table 7). Of the infected children, 7 of 9 were male; all were between the ages of 11 and 15 years; nearly half (4/9) had a history of previous *S. haematobium* infection; and 8 of 9 children had at least one potential indicator of malnutrition (low height for age, low weight for age, or low BMI for age).

Table 7: Descriptive characteristics of the 9 children (2 girls, 7 boys) who were positive for *S. haematobium* eggs in 2010, post-intervention.

	S. haematobium positive children in 2010								
Assigned Case Number	1	2	3	4	5	6	7	8	9
Age as of 2010	10	12	11	15	15	12	13	14	11
Sex	Female	Male	Male	Male	Male	Male	Female	Male	Male
Distance between home and Tini River	Medium	Far	Unknown	Medium	Unknown	Far	Close	Far	Unknown
Total minutes observed using the river	0	0	0	330	0	0	475	0	0
% of days attended school in 2010	0.87	0.97	0.59	0.87	0.91	0.93	0.92	0.96	0.97
New resident of Adasawase as of 2009	1	0	0	1	1	0	0	0	0
Number of times screened in 2010	4	3	3	4	4	4	4	4	4
Low height for age in 2009	n/a	1	1	1	1	n/a	n/a	1	0
Low height for age in 2010	1	1	1	1	1	1	0	1	0
Low weight for age in 2009	1	0	1	1	1	1	1	1	0
Low weight for age in 2010	1	1	1	1	0	1	0	1	0
Low BMI for age in 2009	n/a	0	0	1	0	n/a	n/a	0	0
Low BMI for age in 2010	0	0	0	0	0	1	0	0	0
Egg-positive in 2008	n/a	No	Yes	n/a	n/a	Yes	No	No	Yes
Number of times screened in 2009	4	4	4	3	4	4	4	4	4
Egg-positive in 2009	Yes	No	No	No	No	Yes	Yes	No	No
Treated in 2009	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tested after praziquantel in 2009	Yes	No	No	No	No	Yes	Yes	No	No
Egg-positive after praziquantel in 2009	No	n/a	n/a	n/a	n/a	No	No	n/a	n/a

The risk factors shown in Table 7 suggest demographic and behavioral characteristics that may be associated with infection. However, it is not possible to conclusively state why each child was infected in the presence of the WRA, given the small number of children who were positive.

In this study, schistosomiasis incidence decreased in the presence of the WRA. It is possible that incidence decreased for a reason not related to the pool, although to our knowledge, no other relevant community-wide changes took place during the course of the study. The WRA should be tested further in other communities as behavior change may be location-specific and there was some loss to follow-up due to graduation from the local junior high school and migration. The study shows a biologically relevant and statistically significant decrease in *S. haematobium* incidence in a community in the presence of a WRA; this decrease was not achieved via MDA alone in the year prior to the infrastructure intervention.

Conclusions

Infection incidence decreased significantly among both girl and boy children in the presence of a WRA. Risk factors associated with infection also changed significantly during the course of the study. With respect to children screened in 2008, increasing age was a marginally significant risk factor for infection and male sex and the number of minutes a child was observed to use the Tini River in 2009 were significantly associated with increased risk of infection. In 2009, the only factors associated with infection, given that a child was negative for *S. haematobium* at baseline in 2008, were the number of minutes a child used the Tini River and previous positive infection status. Being male was marginally associated with increased risk of infection ($p = 0.051$) (2009 cohort). The effect of sex on infection risk varies by location (Hammad et al. 1997, Hatz et al.

1998, Ndyomugyenyi and Minjas 2001, Rudge et al. 2008, Satayathum et al. 2006) and is probably related to social norms and not biological susceptibility.

The WRA assessed here should be constructed and evaluated in other water-rich regions to determine whether it is effective in other settings and whether the effect it produces can be sustained by communities over time. Continued mass drug administration (MDA) is still necessary for morbidity control and should be promoted in conjunction with WRAs. In particular, MDA and WRAs should be combined in settings where recreational contact with water is a major risk factor for *S. haematobium* infection. Finally, presumptive treatment of individuals who (a) are frequently observed using contaminated water bodies or (b) were positive previously for *S. haematobium* are potential strategies to combat morbidity and to reduce transmission of *S. haematobium*.

References

1. Bavia, M.E., Malone, J.B., Hale, L., Dantas, A., Marroni, L., Reis, R., 2001. Use of thermal and vegetation index data from earth observing satellites to evaluate the risk of schistosomiasis in Bahia, Brazil. Acta Tropica 79, 79-85.
2. Bethony, J., Williams, J.T., Brooker, S., Gazzinelli, A., Gazzinelli, M.F., LoVerde, P.T., Corrêa Oliveira, R., Kloos, H., 2004. Exposure to Schistosoma mansoni infection in a rural area in Brazil. Part III: household aggregation of water contact behaviour. Tropical Medicine & International Health 9, 381-389.
3. Bethony, J., Williams, J.T., Kloos, H., Blangero, J., Alves Fraga, L., Buck, G., Michalek, A., Williams Blangero, S., LoVerde, P.T., Corréa Oliveira, R., 2001. Exposure to Schistosoma mansoni infection in a rural area in Brazil. II: household risk factors. Tropical Medicine & International Health 6, 136-145.
4. Brooker, S., 2007. Spatial epidemiology of human schistosomiasis in Africa: risk models, transmission dynamics and control. Transactions of the Royal Society of Tropical Medicine and Hygiene 101, 1-8.
5. Brooker, S., Hay, S.I., Bundy, D.A.P., 2002. Tools from ecology: useful for evaluating infection risk models? TRENDS in Parasitology 18, 70-74.
6. Clennon, J.A., King, C.H., Muchiri, E.M., Kariuki, H.C., Ouma, J.H., Mungai, P. and Kitron, U. (2004) "Spatial Patterns of Urinary Schistosomiasis Infection in a Highly Endemic Area of Coastal Kenya." Am. J. Trop. Med Hyg. 70(4): 443-448.

7. Clennon, J.A., Mungai, P.L., Muchiri, E.M., King, C.H., Kitron, U., 2006. Spatial and temporal variations in local transmission of *Schistosoma haematobium* in Msambweni, Kenya. The American Journal of Tropical Medicine and Hygiene 75, 1034-1041.
8. Hammad, T.A., Gabr, N.S., Hussein, M.H., Orieby, A., Shawky, E., Strickland, G.T., 1997. Determinants of infection with schistosomiasis haematobia using logistic regression. The American Journal of Tropical Medicine and Hygiene 57, 464-468.
9. Handzel, T., Karanja, D.M., Addiss, D.G., Hightower, A.W., Rosen, D.H., Colley, D.G., Andove, J., Slutsker, L., and Secor, W.E. (2003) "Geographic distribution of schistosomiasis and soil-transmitted helminths in Western Kenya: implications for anthelminthic mass treatment." Am. J. Trop. Med. Hyg., 69(3): 318 – 323.
10. King, C.H. (2010) "Parasites and poverty: The case of schistosomiasis." Acta Trop., 113: 95 – 104.
11. King, C.H., 2005. Quantification of disease burden due to schistosomiasis, Scientific Working Group on Schistosomiasis. Special Programme for Research and Training in Tropical Diseases (TDR), TDR/SWG/07, Geneva.
12. Kosinski, K.C., Bosompem, K.M., Stadecker, M.J., Wagner, A.D., Plummer, J., Durant, J.L., Gute, D.M., 2011. Diagnostic Accuracy of Urine Filtration and Dipstick Tests for *Schistosoma haematobium* Infection in a Lightly-Infected Population of Ghanaian Schoolchildren. Acta Tropica 118, 123-127.
13. Nsowah-Nuamah, N.N.N., Mensah, G., Aryeetey, M.E., Wagatsuma, Y., Bentil, G., 2001. Urinary schistosomiasis in southern Ghana: a logistic regression approach to data from a community-based integrated control program. The American Journal of Tropical Medicine and Hygiene 65, 484-490.

14. Pereira, W.R., Kloos, H., Crawford, S.B., Velásquez-Melendez, J.G., Matoso, L.F., Fujiwara, R.T., Cançado, G.G.L., LoVerde, P.T., Correa-Oliveira, R., Gazzinelli, A., *Schistosoma mansoni* infection in a rural area of the Jequitinhonha Valley, Minas Gerais, Brazil: Analysis of exposure risk. Acta Tropica 113, 34-41.
15. Rudge, J.W., Stothard, J.R., Basáñez, M.G., Mgeni, A.F., Khamis, I.S., Khamis, A.N., Rollinson, D. (2008) "Micro-epidemiology of urinary schistosomiasis in Zanzibar: local risk factors associated with distribution of infections among schoolchildren and relevance for control." Acta Trop., 105: 45 – 54.
16. Satayathum, SA, Muchiri EM, Ouma JH, Whalen CC, King CH. Factors affecting infection or reinfection with *Schistosoma haematobium* in coastal Kenya: survival analysis during a nine-year, school-based treatment program. *Am J Trop Med Hyg* 2006;75:83-92.
17. Sow S, De Vlas SJ, Mbaye A, Polman K, Gryseels B. Low awareness of intestinal schistosomiasis in northern Senegal after 7 years of health education as part of intense control and research activities. *Trop Med Int Health* 2003; 8: 744–49.
18. Steinmann, P., Keiser, J., Bos, R., Tanner, M., and Utzinger, J. (2006) "Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk." Lancet Infect. Dis., 6: 411 – 425.
19. Stothard, J.R., French, M.D., Simba Khamis, I., Basáñez, M.G., Rollinson, D., 2009. "The epidemiology and control of urinary schistosomiasis and soil-transmitted helminthiasis in schoolchildren on Unguja Island, Zanzibar." Transactions of the Royal Society of Tropical Medicine and Hygiene 103, 1031-1044.
20. van der Werf, M.J., de Vlas, S.J., Brooker, S., Looman, C.W.N., Nagelkerke, N.J.D., Habbema, J.D.F., Engels, D., 2003. Quantification of clinical morbidity associated with

schistosome infection in sub-Saharan Africa. Acta Tropica 86, 125-139.

21. World Health Organization, 1993. The control of schistosomiasis: Second report of the WHO Expert Committee, 2 ed. World Health Organization, Geneva.

CHAPTER 6: CONCLUSIONS & RECOMMENDATIONS

Summary of Chapter 1 Literature Review

Schistosomiasis is a neglected tropical disease caused by parasitic worms in the genus *Schistosoma*; infection is common in sub-Saharan Africa. Schistosomes of three different species, *S. haematobium*, *S. mansoni*, and *S. japonicum*, affect between 207 million and 587 million people in 76 countries (Engels et al. 2002, King 2010, Steinmann et al. 2006). Infection is transmitted via skin contact with water contaminated by human waste. Pathology for urogenital schistosomiasis, caused by *S. haematobium*, may result from chronic or acute infection and ranges from hematuria and dysuria to bladder cancer and death. There are a variety of risk factors that increase the probability of contracting schistosomiasis, and correspondingly, a variety of options to control transmission and resulting morbidity. The main goal of this thesis was to assess *S. haematobium* infection incidence in the absence and presence of infrastructure designed to reduce water contact. Specifically, a water recreation area (WRA) was evaluated for its ability to reduce *S. haematobium* incidence in Adasawase, a rural community in the Eastern Region of Ghana (approximate population: 2,000 people). The water recreation area described in the preceding chapters should be equally effective at preventing schistosomiasis caused by all three main types of schistosomes, given that infection route, namely dermal contact with contaminated water, is common to all species.

Summary of Chapter 2 Hypothesis and Objectives

The study community, Adasawase, and the study population were described and the rationale for conducting the study was explained. The chief of Adasawase invited our study team to screen

school-aged children for *S. haematobium* and to collaboratively design a sustainable solution to the problem of infection.

Also in Chapter 2, the thesis hypothesis was presented. A water recreation area was designed and implemented and *S. haematobium* incidence rates were compared before and after the water recreation was opened for public use. It was expected that recreational use of Tini River would decrease in the presence of the water recreation area and thus, the number of new *S. haematobium* infections would also decrease. Finally, the study objectives presented in Chapter 2 describe the steps taken to: screen and treat schoolchildren for *S. haematobium* before and after implementing a water recreation area; design, construct, and operate a water recreation area; observe behavior at the Tini River with respect to water use practices; and statistically analyze data collected during the study.

Summary of Chapter 3 Sensitivity and Specificity of Two Diagnostic Tests

In Chapter 3, data are presented that show the sensitivity of a filtration technique commonly used to detect *S. haematobium* eggs in urine. In addition, the sensitivity and specificity of a semi-quantitative dipstick test used to detect hematuria, a proxy for infection, were estimated. These screening tests are frequently used clinically to diagnose infections and also to estimate population-level prevalence of infection and/or morbidity. Despite their frequent use in clinical, academic, or public health settings, the literature did not contain information about the accuracy of these diagnostic tests in a lightly infected population (WHO definition: < 50 eggs/10ml urine) until Chapter 3 was published (WHO 2003b). Filling this knowledge gap is increasingly important in light of mass drug administration (MDA). Following MDA, some populations

contain large numbers of lightly-infected individuals who are difficult to detect with current diagnostic techniques.

Data are presented showing that it is necessary to screen individuals more than one time for *S. haematobium* eggs and/or hematuria. Ideally, at least three screenings per person should be conducted when urine samples are negative. The mean sensitivity of a single filtration test for *S. haematobium* eggs among the population studied was 56.1% for girls and 62.3% for boys. The mean was calculated as the arithmetic mean of three calculated sensitivities for the three separate screenings per person. With respect to the semi-quantitative dipstick test for hematuria, the specificity remains high (> 80%) even when children are screened 3 times. Performing a dipstick test 3 times on a single child in Adasawase resulted in an overall sensitivity of 68.2% for girls and 70.6% for boys. These findings have important clinical, academic, and public health implications.

Summary of Chapter 4 Description of Water Recreation Area Design and Construction

A description is presented of the design and construction of a novel water recreation area (WRA) that was implemented to reduce *S. haematobium* infection rates in Adasawase, Ghana. The primary prevention of infection (transmission control) has received little attention to date, despite the fact that reinfection with schistosomes is common after treatment. Mass drug administration (MDA) is currently the strategy of choice control of schistosomiasis, but the focus is mainly morbidity control, as opposed to transmission control.

In collaboration with the community of Adasawase, a WRA was designed to meet the needs of the target population (schoolchildren), to serve as an alternative to the use of a contaminated local river, and to be both cost-effective and sustainable. The only consumable

material required for operation is water; the regular input of labor is also necessary. The WRA is filled with water from two sources: groundwater that is hand-pumped into the pool from a pre-existing borehole, and rainwater from a rooftop collection system. The entire facility was constructed from locally-available materials and has an expected lifespan of over 30 years. The WRA opened for public use in July 2009 and has been operated and maintained by the community since that time. It is important to note that this intervention is appropriate only for water-rich areas.

Summary of Chapter 5 Evaluation of the Efficacy of the Water Recreation Area

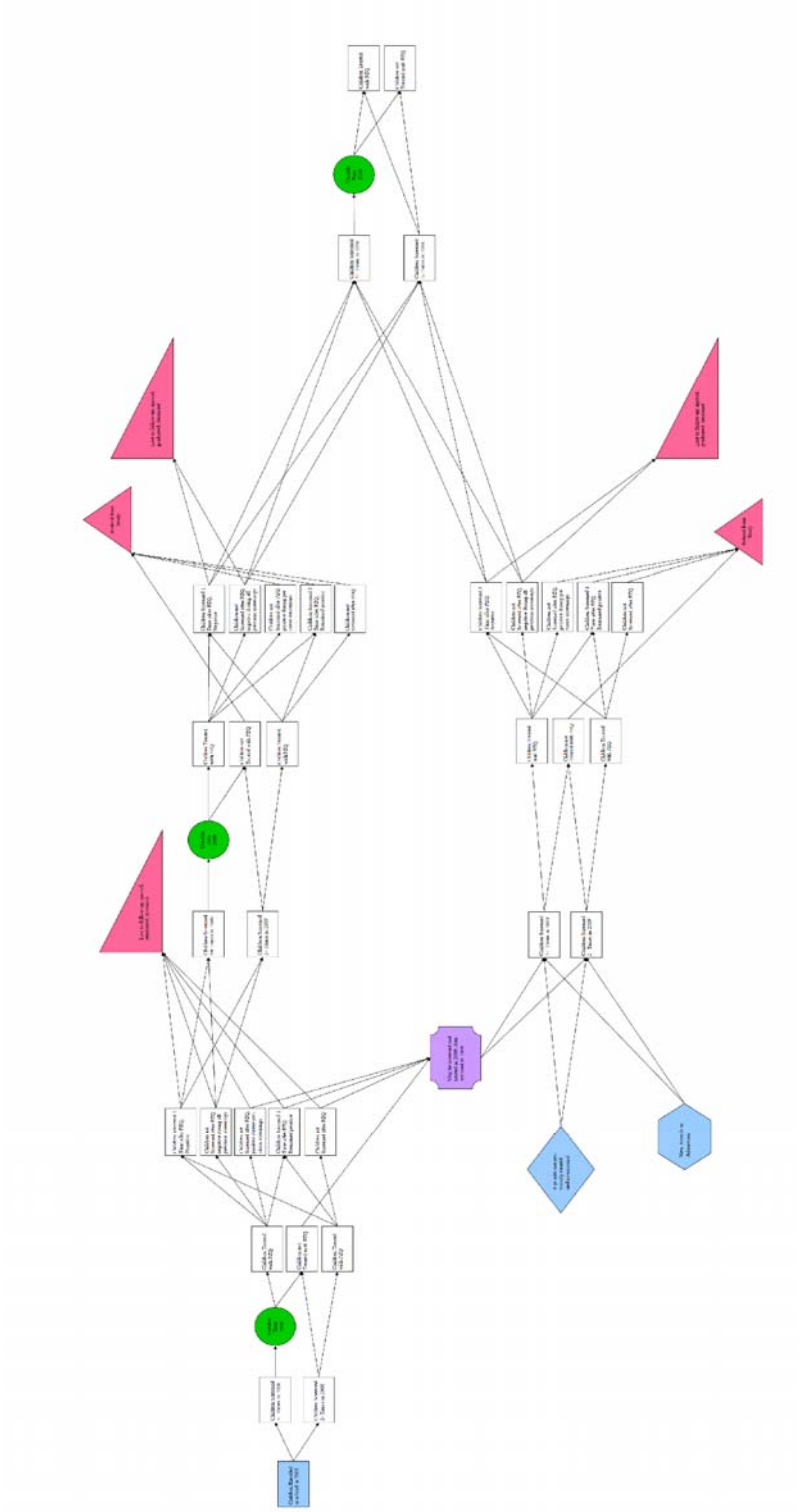
S. haematobium screening of schoolchildren in Adasawase was conducted in 2008, 2009, and 2010. In collaboration with Ghana Health Services, treatment with praziquantel was provided annually. A WRA was opened for public use in 2009; design and construction are detailed in Chapter 4. Incidence of infection pre-intervention was compared with incidence of infection post-intervention. In 2008, infection prevalence (via filtration for *S. haematobium* eggs) was 36.1% among girls and 50.7% among boys. After praziquantel treatment, children were rescreened to ensure parasite clearance. In 2009, in the absence of the WRA, annual incidence of infection was 13.3% among girls and 23.8% among boys. Praziquantel was again administered and children were rescreened to ensure *S. haematobium* clearance. In 2010, in the presence of the WRA, annual incidence was 1.9% among girls and 5.0% among boys.

In addition to collecting data about infection status, data were also collected about: water contact behavior at the Tini River; school attendance for the year 2009-2010; home location; and nutritional indicators, such as height, weight, and body mass index for age. Risk factors for infection in 2008 and 2009 were assessed for statistical significance via logistic regression

analysis. Due to the fact that only 9 children in the study were *S. haematobium* positive in 2010, further analysis such as logistic regression was not performed. Instead, characteristics of each child are presented. No single characteristic universally explains infection status, but 7 of the 9 children were boys and all were between the ages of 11 and 15 years.

The WRA is a promising method for preventing *S. haematobium* infection. Use of infrastructure to reduce *S. haematobium* infection prevalence will: (a) decrease reliance on mass drug administration strategies; (b) empower communities to address public health concerns about *S. haematobium*; and (c) reduce the morbidity and mortality experienced by individuals who lack access to regular treatment. The WRA should be implemented and evaluated in additional water-rich communities where recreational contact with water is a major risk factor for infection with schistosomes. Mass drug administration remains necessary for morbidity control and should be promoted in conjunction with primary prevention techniques.

APPENDIX A



REFERENCES

1. Allen, H., Crompton, D. W. T., de Silva, N., LoVerde, P. T., & Olds, G. R. (2002). New policies for using anthelmintics in high risk groups. *Trends in Parasitology, 18*(9), 381-382.
2. Asaolu, S. O., & Ofoezie, I. E. (1990). A simple method for concentrating eggs of Schistosoma haematobium in the urine. *Nigerian Journal of Parasitology, 9*(11), 47–50.
3. Bagley, S. C., White, H., & Golomb, B. A. (2001). Logistic regression in the medical literature: Standards for use and reporting, with particular attention to one medical domain. *Journal of Clinical Epidemiology, 54*(10), 979-985.
4. Bartram, J. (2008). Flowing away: Water and health opportunities. *Bulletin of the World Health Organization, 86*, 2-3.
5. Bethony, J., Williams, J. T., Kloos, H., Blangero, J., Alves Fraga, L., Buck, G., et al. (2001). Exposure to Schistosoma mansoni infection in a rural area in Brazil. II: household risk factors. *Tropical Medicine & International Health, 6*(2), 136-145.
6. Bewick, V., Cheek, L., & Ball, J. (2005). Statistics review 14: Logistic regression. *Critical Care, 9*(1), 112.
7. Birrie, H., Medhin, G., & Jemaneh, L. (1995). Comparison of urine filtration and a chemical reagent strip in the diagnosis of urinary schistosomiasis in Ethiopia. *East African Medical Journal, 72*(3), 180-185.
8. Bosompem, K. M. (2008). Senior Parasitologist, Noguchi Memorial Institute for Medical Research, University of Ghana. Legon.

9. Bosompem, K. M., Asigbee, J., Otchere, J., Haruna, A., Kpo, K. H., & Kojima, S. (1998). Accuracy of diagnosis of urinary schistosomiasis: Comparison of parasitological and a monoclonal antibody-based dipstick method. *Parasitology International, 47*(3), 211-217.

10. Bosompem, K. M., Bentum, I. A., Otchere, J., Anyan, W. K., Brown, C. A., Osada, Y., et al. (2004). Infant schistosomiasis in Ghana: a survey in an irrigation community. *Tropical Medicine & International Health, 9*(8), 917-922.

11. Bosompem, K. M., Owusu, O., Okanla, E. O., & Kojima, S. (2004). Applicability of a monoclonal antibody based dipstick in diagnosis of urinary schistosomiasis in the Central Region of Ghana. *Tropical Medicine & International Health, 9*(9), 991-996.

12. Bosu, W. K., Ahelegbe, D., Edum-Fotwe, E., Kobina, A. B., & Kobina Turkson, P. (1997). Factors influencing attendance to immunization sessions for children in a rural district of Ghana. *Acta Tropica, 68*(3), 259-267.

13. Bradley, D. J., & McCullough, F. S. (1973). Egg output stability and the epidemiology of Schistosoma haematobium Part II. An analysis of the epidemiology of endemic S. haematobium. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 67*(4), 491-500.

14. Bruun, B., & Aagaard-Hansen, J. (14 – 16 November 2005). *The sociocultural context of schistosomiasis control: current knowledge and future research needs.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

15. Cairncross, S. (2003). Editorial: Water supply and sanitation: some misconceptions. *Tropical Medicine & International Health, 8*(3), 193-195.

16. Cairncross, S., & Feachem, R. (1993). *Environmental Health Engineering in the Tropics* (2 ed.). New York: John Wiley & Sons.

17. Cairncross, S., & Kinnear, J. (1991). Water vending in urban Sudan. *International Journal of Water Resources Development, 7*(4), 267-273.

18. Centers for Disease Control, U. S. o. A. (2011). www.cdc.gov. Retrieved 3 August 2011, 2011

19. Chenine, A. L., Shai-Kobiler, E., Steele, L. N., Ong, H., Augostini, P., Song, R., et al. (2008). Acute Schistosoma mansoni infection increases susceptibility to systemic SHIV clade C infection in rhesus macaques after mucosal virus exposure. *PLoS Neglected Tropical Diseases, 2*(7), e265.

20. Chitsulo, L., Engels, D., Montresor, A., & Savioli, L. (2000). The global status of schistosomiasis and its control. *Acta Tropica, 77*(1), 41-51.

21. Cioli, D. (2005). *Research towards new treatment for schistosomiasis*. Paper presented at the Scientific Working Group on Schistosomiasis.

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

23. Clennon, J. A., Mungai, P. L., Muchiri, E. M., King, C. H., & Kitron, U. (2006). Spatial and temporal variations in local transmission of *Schistosoma haematobium* in Msambweni, Kenya. *The American Journal of Tropical Medicine and Hygiene, 75*(6), 1034-1041.

24. Coon, D. R. (2005). Schistosomiasis: Overview of the history, biology, clinicopathology, and laboratory diagnosis. *Clinical Microbiology Newsletter, 27*(21), 163-168.

25. Danso-Appiah, A., De Vlas, S. J., Bosompem, K. M., & Habbema, J. D. F. (2004). Determinants of health seeking behaviour for schistosomiasis related symptoms in the

context of integrating schistosomiasis control within the regular health services in Ghana. *Tropical Medicine & International Health, 9*(7), 784-794.

26. De Vlas, S. J., Danso-Appiah, A., van der Werf, M. J., Bosompem, K. M., & Habbema, J. D. F. (2004). Quantitative evaluation of integrated schistosomiasis control: the example of passive case finding in Ghana. *Tropical Medicine & International Health, 9*(6), A16-A21.

27. Doehring, E., Vester, U., Ehrich, J. H. H., & Feldmeier, H. (1985). Circadian variation of ova excretion, proteinuria, hematuria, and leukocyturia in urinary schistosomiasis. *Kidney International, 27*(4), 667-671.

28. Doumenge, J. P. (1987). *Atlas of the global distribution of schistosomiasis*: Presses Universitaires de Bordeaux Bordeaux, France.

29. El-Katsha, S., & Watts, S. (2002). *Gender, behavior, and health: Schistosomiasis transmission and control in rural Egypt*: American University in Cairo Press.

30. Eltoum, I. A., Sulaiman, S., Ismail, B. M., Ali, M. M. M., Elfatih, M., & Homeida, M. (1992). Evaluation of haematuria as an indirect screening test for schistosomiasis haematobium: a population-based study in the White Nile province, Sudan. *Acta Tropica, 51*(2), 151-157.

31. Engels, D., Chitsulo, L., Montresor, A., & Savioli, L. (2002). The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Tropica, 82*(2), 139-146.

32. Feldmeier, H., Doehring, E., Daffala, A. A., Omer, A. H. S., & Dietrich, M. (1982). Efficacy of metrifonate in urinary schistosomiasis: comparison of reduction of

Schistosoma haematobium and *S. mansoni* eggs. *The American Journal of Tropical Medicine and Hygiene, 31*(6), 1188-1194.

33. Feldmeier, H., Doehring, E., & Daffalla, A. A. (1982). Simultaneous use of a sensitive filtration technique and reagent strips in urinary schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 76*(3), 416-421.

34. Feldmeier, H., & Poggensee, G. (1993). Diagnostic techniques in schistosomiasis control. A review. *Acta Tropica, 52*(4), 205-220.

35. French, M. D., Rollinson, D., Basáñez, M. G., Mgeni, A. F., Khamis, I., & Stothard, J. R. (2007). School-based control of urinary schistosomiasis on Zanzibar, Tanzania: monitoring micro-haematuria with reagent strips as a rapid urological assessment. *Journal of Pediatric Urology, 3*(5), 364-368.

36. Friedman, J. F., Kurtis, J. D., McGarvey, S. T., Fraga, A. L., Silveira, A., Pizziolo, V., et al. (2001). Comparison of self-reported and observed water contact in an S. mansoni endemic village in Brazil. *Acta Tropica, 78*(3), 251-259.

37. Gause, W. C., Urban, J. F., & Stadecker, M. J. (2003). The immune response to parasitic helminths: insights from murine models. *Trends in Immunology, 24*(5), 269-277.

38. Gazzinelli, A., Bethony, J., Fraga, L. A., LoVerde, P. T., Correa Oliveira, R., & Kloos, H. (2001). Exposure to Schistosoma mansoni infection in a rural area of Brazil. I: Water contact. *Tropical Medicine & International Health, 6*(2), 126-135.

39. Geary, T. G., Woo, K., McCarthy, J. S., Mackenzie, C. D., Horton, J., Prichard, R. K., et al. (2010). Unresolved issues in anthelmintic pharmacology for helminthiases of humans. *International Journal for Parasitology, 40*(1), 1-13.

40. Goodman, D., Haji, H. J., Bickle, Q. D., Stoltzfus, R. J., Tielsch, J. M., Ramsan, M., et al. (2007). A comparison of methods for detecting the eggs of *Ascaris*, *Trichuris*, and hookworm in infant stool, and the epidemiology of infection in Zanzibari infants. *The American Journal of Tropical Medicine and Hygiene, 76*(4), 725-731.

41. Gordon, W. S. (2006). *Schistosomiasis treatment and control in Africa: Who gets treated and who gets missed?* , Harvard University, Boston, Massachusetts.

42. Gryseels, B., Polman, K., Clerinx, J., & Kestens, L. (2006). Human schistosomiasis. *The Lancet, 368*(9541), 1106-1118.

43. Guyatt, H. (2003). The cost of delivering and sustaining a control programme for schistosomiasis and soil-transmitted helminthiasis. *Acta Tropica, 86*(2-3), 267-274.

44. Guyatt, H., Brooker, S., Lwambo, N. J. S., Siza, J. E., & Bundy, D. A. P. (1999). The performance of school based questionnaires of reported blood in urine in diagnosing *Schistosoma haematobium* infection: patterns by age and sex. *Tropical Medicine & International Health, 4*(11), 751-757.

45. Guyatt, H., Evans, D., Lengeler, C., & Tanner, M. (1994). Controlling schistosomiasis: the cost-effectiveness of alternative delivery strategies. *Health Policy and Planning, 9*(4), 385-395.

46. Guyatt, H., & Tanner, M. (1996). Different approaches to modeling the cost effectiveness of schistosomiasis control. *The American Journal of Tropical Medicine and Hygiene, 55*(5), 159-164.

47. Gyorkos, T. W., Ramsan, M., Foum, A., & Khamis, I. S. (2001). Efficacy of new low-cost filtration device for recovering Schistosoma haematobium eggs from urine. *Journal of Clinical Microbiology, 39*(7), 2681-2682.

48. Hall, A. (1982). Intestinal helminths of man: the interpretation of egg counts. *Parasitology, 85*(03), 605-613.

49. Hammad, T. A., Gabr, N. S., Hussein, M. H., Orieby, A., Shawky, E., & Strickland, G. T. (1997). Determinants of infection with schistosomiasis haematobia using logistic regression. *The American Journal of Tropical Medicine and Hygiene, 57*(4), 464-468.

50. Handzel, T., Karanja, D., Addiss, D. G., Hightower, A. W., Rosen, D. H., Colley, D. G., et al. (2003). Geographic distribution of schistosomiasis and soil-transmitted helminths in Western Kenya: implications for anthelminthic mass treatment. *The American Journal of Tropical Medicine and Hygiene, 69*(3), 318-323.

51. Harms, G., & Feldmeier, H. (2005). The impact of HIV infection on tropical diseases. *Infectious Disease Clinics of North America, 19*(1), 121-135.

52. Hartgers, F. C., Obeng, B. B., Boakye, D., & Yazdanbakhsh, M. (2008). Immune responses during helminth-malaria co-infection: a pilot study in Ghanaian school children. *Parasitology, 135*(07), 855-860.

53. Hartgers, F. C., & Yazdanbakhsh, M. (2006). Co infection of helminths and malaria: modulation of the immune responses to malaria. *Parasite Immunology, 28*(10), 497-506.

54. Hatz, C., Savioli, L., Mayombana, C., Dhunputh, J., Kisumku, U. M., & Tanner, M. (1990). Measurement of schistosomiasis-related morbidity at community level in areas of different endemicity. *Bulletin of the World Health Organization, 68*(6), 777-787.

55. Hatz, C. F., Vennervald, B. J., Nkulila, T., Vounatsou, P., Kombe, Y., Mayombana, C., et al. (1998). Evolution of Schistosoma haematobium-related pathology over 24 months after treatment with praziquantel among school children in southeastern Tanzania. *The American Journal of Tropical Medicine and Hygiene, 59*(5), 775-781.

56. Hirayama, K. (14 - 16 November 2005). *Schistosomiasis and immunity.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

57. Hopkins, O. S., Lauria, D. T., & Kolb, A. (2004). Demand-based planning of rural water systems in developing countries. *Journal of Water Resources Planning and Management, 130*(1), 44-52.

58. Hosmer, D. W., & Lemeshow, S. (1989). *Applied Logistic Regression* (1 ed.). New York: John Wiley & Sons.

59. Hotez, P. J., Bundy, D. A. P., Beegle, K., Brooker, S., Drake, L., De Silva, N., et al. (2006). Helminth infections: Soil-transmitted helminth infections and schistosomiasis. In D. Jamison, J. T. Bremen, A. R. Measham, G. Alleyne, M. Claeson, D. B. Evans, P. Jha, A. Mills & P. Musgrove (Eds.), *Disease Control Priorities in Developing Countries* (2 ed., pp. 1394). New York: Oxford University Press.

60. Husting, E. L. (1970). Sociological patterns and their influence on the transmission of Bilharziasis. *The Central African Journal of Medicine, Supplement, July*, 5-10.

61. Kabatereine, N. B., Brooker, S., Koukounari, A., Fleming, F., Tukahebwa, E. M., Kazibwe, F., et al. (2005). *Implementation strategies for schistosomiasis control in Uganda, and research needs under the programme.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

62. Katsivo, M. N., Muthami, L. N., Karama, M., & Kingori, F. (1993a). Perception of a schistosomiasis control project in rural Kenya by the beneficiaries. *East African Medical Journal, 70*(10), 613-616.

63. Katsivo, M. N., Muthami, L. N., Kimani, S., Karama, M., & Kingori, F. (1993b). Involvement of a community in schistosomiasis control: a Kenyan experience. *East African Medical Journal, 70*(8), 478-481.

64. King, C. H. (14 - 16 November 2005). *Quantification of disease burden due to schistosomiasis.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

65. King, C. H. (2009). Toward the elimination of schistosomiasis. *New England Journal of Medicine, 360*(2), 106-109.

66. King, C. H. (2010). Parasites and poverty: the case of schistosomiasis. *Acta Tropica, 113*(2), 95-104.

67. King, C. H., Blanton, R. E., Muchiri, E. M., Ouma, J. H., Kariuki, H. C., Mungai, P., et al. (2004). Low heritable component of risk for infection intensity and infection-associated disease in urinary schistosomiasis among Wadigo village populations in Coast Province, Kenya. *The American Journal of Tropical Medicine and Hygiene, 70*(1), 57.

68. King, C. H., Dickman, K., & Tisch, D. J. (2005). Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *The Lancet, 365*(9470), 1561-1569.

69. King, C. H., Sturrock, R. F., Kariuki, H. C., & Hamburger, J. (2006). Transmission control for schistosomiasis-why it matters now. *Trends in Parasitology, 22*(12), 575-582.

70. Kleemeier, E. (2000). The impact of participation on sustainability: an analysis of the Malawi rural piped scheme program. *World Development, 28*(5), 929-944.

71. Kloetzel, K. (1992). Some personal views on the control of schistosomiasis mansoni. *Memórias do Instituto Oswaldo Cruz, 87*, 221-226.

72. Kloos, H., Ouma, J., Kariuki, H. C., & Butterworth, A. E. (1986). Knowledge, perceptions and health behavior pertaining to Schistosoma mansoni related illness in Machakos district, Kenya. *Tropical Medicine and Parasitology, 37*(2), 171.

73. Korkor Ansah, E., Narh-Bana, S., Asiamah, S., Dzordzordzi, V., Biantey, K., Dickson, K., et al. (2009). Effect of removing direct payment for health care on utilisation and health outcomes in Ghanaian children: a randomised controlled trial. *PLoS Medicine, 6*(1), e1000007.

74. Koukounari, A., Webster, J. P., Donnelly, C. A., Bray, B. C., Naples, J., Bosompem, K., et al. (2009). Sensitivities and Specificities of Diagnostic Tests and Infection Prevalence of Schistosoma haematobium Estimated from Data on Adults in Villages Northwest of Accra, Ghana. *The American Journal of Tropical Medicine and Hygiene, 80*(3), 435-441.

75. Kvalsvig, J. D., & Schutte, C. H. J. (1986). The role of human water contact patterns in the transmission of schistosomiasis in an informal settlement near a major industrial area. *Annals of Tropical Medicine and Parasitology, 80*(1), 13-26.

76. Lambertucci, J. R. (14 - 16 November 2005). *Clinical schistosomiasis.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

77. Lancet. (2004). Thinking beyond deworming. [Editorial]. *The Lancet, 364*, 1993-1994.

78. Lengeler, C., Kilima, P., Mshinda, H., Morona, D., Hatz, C., & Tanner, M. (1991). Rapid, low-cost, two-step method to screen for urinary schistosomiasis at the district level: the Kilosa experience. *Bulletin of the World Health Organization, 69*(2), 179-189.

79. Lima e Costa, M. F. F., Magalhães, M. H. A., Rocha, R. S., Antunes, C. M. F., & Katz, N. (1987). Water-contact patterns and socioeconomic variables in the epidemiology of

schistosomiasis mansoni in an endemic area in Brazil. *Bulletin of the World Health Organization, 65*(1), 57.

80. Loker, E. S. (14 - 16 November 2005). *Research on the molluscan intermediate hosts of schistosomiasis: What are the priorities?* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

81. Mafiana, C. F., Ekpo, U. F., & Ojo, D. A. (2003). Urinary schistosomiasis in preschool children in settlements around Oyan Reservoir in Ogun State, Nigeria: implications for control. *Tropical Medicine & International Health, 8*(1), 78-82.

82. Magnussen, P., Muchiri, E., Mungai, P., Ndzovu, M., Ouma, J., & Tosha, S. (1997). A school based approach to the control of urinary schistosomiasis and intestinal helminth infections in children in Matuga, Kenya: impact of a two year chemotherapy programme on prevalence and intensity of infections. *Tropical Medicine & International Health, 2*(9), 825-831.

83. McCullough, F. S., & Bradley, D. J. (1973). Egg output stability and the epidemiology of Schistosoma haematobium. I. Variation and stability in Schistosoma haematobium egg counts. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 67*(4), 475-490.

84. McGarvey, S. T. (14 - 16 November 2005). *Ecological and other factors related to schistosomiasis.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

85. Molyneux, D. H., Hotez, P. J., & Fenwick, A. (2005). "Rapid-impact interventions": how a policy of integrated control for Africa's neglected tropical diseases could benefit the poor. *PLoS Medicine, 2*(11), e336.

86. Mott, K. E. (1982). A reusable polyamide filter for diagnosis of S. haematobium infection by urine filtration. *Bulletin de la Société de Pathologie Exotique et de ses Filiales, 76*(1), 101-104.

87. Mott, K. E., Dixon, H., Osei-Tutu, E., England, E. C., Ekue, K., & Tekle, A. (1985). Indirect screening for *Schistosoma haematobium* infection: a comparative study in Ghana and Zambia. *Bulletin of the World Health Organization, 63*(1), 135-142.

88. Mshinda, H., Lengeler, C., Hatz, C., & de Savigny, D. (1989). Field diagnosis of urinary schistosomiasis by multiple use of nuclepore urine filters. *The Journal of Parasitology, 75*(3), 476-478.

89. Ndyomugyenyi, R., & Kabatereine, N. (2003). Integrated community directed treatment for the control of onchocerciasis, schistosomiasis and intestinal helminths infections in Uganda: advantages and disadvantages. *Tropical Medicine & International Health, 8*(11), 997-1004.

90. Ndyomugyenyi, R., & Minjas, J. N. (2001). Urinary schistosomiasis in schoolchildren in Dar-es-Salaam, Tanzania, and the factors influencing its transmission. *Annals of Tropical Medicine and Parasitology, 95*(7), 697-706.

91. Nsowah-Nuamah, N. N. N., Aryeetey, M. E., Jolayemi, E. T., Wagatsuma, Y., Mensah, G., Dontwi, I. K., et al. (2004). Predicting the timing of second praziquantel treatment and its effect on reduction of egg counts in southern Ghana. *Acta Tropica, 90*(3), 263-270.

92. Nsowah-Nuamah, N. N. N., Mensah, G., Aryeetey, M. E., Wagatsuma, Y., & Bentil, G. (2001). Urinary schistosomiasis in southern Ghana: a logistic regression approach to data from a community-based integrated control program. *The American Journal of Tropical Medicine and Hygiene, 65*(5), 484-490.

93. Oladejo, S. O., & Ofoezie, I. E. (2006). Unabated schistosomiasis transmission in Erinle River Dam, Osun State, Nigeria: evidence of neglect of environmental effects of development projects. *Tropical Medicine & International Health, 11*(6), 843-850.

94. Olds, G. R. (14 - 16 November 2005). *Schistosomiasis in women of childbearing age, including in pregnancy.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

95. Opara, K. N., Udoidung, N. I., & Ukpong, I. G. (2007). Genitourinary schistosomiasis among pre-primary schoolchildren in a rural community within the Cross River Basin, Nigeria. *Journal of Helminthology, 81*(04), 393-397.

96. Pereira, W. R., Kloos, H., Crawford, S. B., Velásquez-Melendez, J. G., Matoso, L. F., Fujiwara, R. T., et al. (2010). *Schistosoma mansoni* infection in a rural area of the Jequitinhonha Valley, Minas Gerais, Brazil: Analysis of exposure risk. *Acta Tropica, 113*(1), 34-41.

97. Peters, P. A., Mahmoud, A. A. F., Warren, K. S., Ouma, J. H., & Siongok, T. K. A. (1976a). Field studies of a rapid, accurate means of quantifying *Schistosoma haematobium* eggs in urine samples. *Bulletin of the World Health Organization, 54*(2), 159-162.

98. Peters, P. A., Warren, K. S., & Mahmoud, A. A. (1976b). Rapid, accurate quantification of schistosome eggs via Nuclepore filters. *The Journal of Parasitology, 62*(1), 154-155.

99. Pitchford, R. J. (1958). Influence of living conditions on bilharziasis infection rates in Africans in the Transvaal. *Bulletin of the World Health Organization, 18*(5-6), 1088.

100. Polderman, A. M. (1984). Cost-effectiveness of different ways of controlling intestinal schistosomiasis: A case study* 1. *Social Science & Medicine, 19*(10), 1073-1080.

101. Pugh, R. N. (1978). A filtration method for schistosome egg quantification. *Annals of Tropical Medicine and Parasitology, 72*(4), 387-388.

102. Rabello, A. a. E., M. (14 - 16 November 2005). *Progress towards the detection of schistosomiasis.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

103. Raso, G., Matthys, B., N'Goran, E. K., Tanner, M., Vounatsou, P., & Utzinger, J. (2005). Spatial risk prediction and mapping of *Schistosoma mansoni* infections among schoolchildren living in western Cote d'Ivoire. *Parasitology, 131*(01), 97-108.

104. Ratard, R. C., Kouemeni, L. E., Bessala, M. M., & Ndamkou, C. N. (1992). Estimation of the number of cases of schistosomiasis in a country: the example of Cameroon. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 86*(3), 274-276.

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

106. Sarpong, N., Loag, W., Fobil, J., Meyer, C. G., Adu Sarkodie, Y., May, J., et al. (2010). National health insurance coverage and socio economic status in a rural district of Ghana. *Tropical Medicine & International Health, 15*(2), 191-197.

107. Satayathum, S. A., Muchiri, E. M., Ouma, J. H., Whalen, C. C., & King, C. H. (2006). Factors affecting infection or reinfection with Schistosoma haematobium in coastal Kenya: survival analysis during a nine-year, school-based treatment program. *The American Journal of Tropical Medicine and Hygiene, 75*(1), 83.

108. Savioli, L., & Dixon, H. (1989). Control of morbidity due to Schistosoma haematobium on Pemba island; selective population chemotherapy of schoolchildren with haematuria to identify high-risk localities. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 83*(6), 805-810.

109. Savioli, L., Hatz, C., Dixon, H., Kisumku, U. M., & Mott, K. E. (1990). Control of morbidity due to *Schistosoma haematobium* on Pemba Island: egg excretion and hematuria as indicators of infection. *The American Journal of Tropical Medicine and Hygiene, 43*(3), 289-295.

110. Schutte, C. H., Pienaar, R., Becker, P. J., Viljoen, E., Crowther, G., van Deventer, J. M., et al. (1994). Observations on the techniques used in the qualitative and quantitative diagnosis of schistosomiasis. *Annals of Tropical Medicine and Parasitology, 88*(3), 305-316.

111. Scott, J. A. (1957). Egg counts as estimates of intensity of infection with Schistosoma haematobium. *Texas Reports on Biology and Medicine, 15*(3), 425.

112. Secor, W. E. (2005). Immunology of human schistosomiasis: off the beaten path. *Parasite Immunology, 27*(7 8), 309-316.

113. Shiff, C., Veltri, R., Naples, J., Quartey, J., Otchere, J., Anyan, W., et al. (2006). Ultrasound verification of bladder damage is associated with known biomarkers of bladder cancer in adults chronically infected with Schistosoma haematobium in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 100*(9), 847-854.

114. Sow, S., Vlas, S. J., Mbaye, A., Polman, K., & Gryseels, B. (2003). Low awareness of intestinal schistosomiasis in northern Senegal after 7 years of health education as part of

intense control and research activities. *Tropical Medicine & International Health, 8*(8), 744-749.

115. Steinmann, P., Keiser, J., Bos, R., Tanner, M., & Utzinger, J. (2006). Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *The Lancet Infectious Diseases, 6*(7), 411-425.

116. Stephenson, L. S., Latham, M. C., Kinoti, S. N., & Oduori, M. L. (1984). Sensitivity and specificity of reagent strips in screening of Kenyan children for *Schistosoma haematobium* infection. *The American Journal of Tropical Medicine and Hygiene, 33*(5), 862-871.

117. Stothard, J. R., French, M. D., Simba Khamis, I., Basáñez, M. G., & Rollinson, D. (2009). The epidemiology and control of urinary schistosomiasis and soil-transmitted helminthiasis in schoolchildren on Unguja Island, Zanzibar. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 103*(10), 1031-1044.

118. Stothard, J. R., & Gabrielli, A. F. (2007). Schistosomiasis in African infants and preschool children: to treat or not to treat? *Trends in Parasitology, 23*(3), 83-86.

119. Stothard, J. R., Mook, P., Mgeni, A. F., Khamis, I. S., Khamis, A. N., & Rollinson, D. (2006). Control of urinary schistosomiasis on Zanzibar (Unguja Island): a pilot evaluation of the educational impact of the Juma na Kichocho health booklet within primary schools. *Memórias do Instituto Oswaldo Cruz, 101*, 119-124.

120. Taylor-Robinson, D., Jones, A., & Garner, P. (2009). Does deworming improve growth and school performance in children? *PLoS Neglected Tropical Diseases, 3*(1), e358.

121. Tchuem Tchuenté, L. A., Southgate, V. R., Webster, B. L., De Bont, J., & Vercruysse, J. (2001). Impact of installation of a water pump on schistosomiasis transmission in a focus

in Cameroon. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 95*, 255-256.

122. Touré, S., Zhang, Y., Bosqué-Oliva, E., Ky, C., Ouedraogo, A., Koukounari, A., et al. (2008). Two-year impact of single praziquantel treatment on infection in the national control programme on schistosomiasis in Burkina Faso. *Bulletin of the World Health Organization, 86*, 780-787.

123. Tucker, J. B. (1983). Schistosomiasis and water projects: breaking the link. *Environment: Science and Policy for Sustainable Development, 25*(7), 17-20.

124. Useh, M. F., & Ejezie, G. C. (1999). Modification of behaviour and attitude in the control of schistosomiasis. I. Observations on water-contact patterns and perception of infection. *Annals of Tropical Medicine and Parasitology, 93*(7), 711-720.

125. Utzinger, J., Bergquist, R., Shu-Hua, P. X., Singer, B. H., & Tanner, P. M. (2003). Sustainable schistosomiasis control-the way forward. *The Lancet, 362*(9399), 1932-1933.

126. Utzinger, J., Booth, M., N'Goran, E. K., Müller, I., Tanner, M., & Lengeler, C. (2001). Relative contribution of day-to-day and intra-specimen variation in faecal egg counts of *Schistosoma mansoni* before and after treatment with praziquantel. *Parasitology, 122*(05), 537-544.

127. van der Werf, M. J., Bosompem, K. M., & de Vlas, S. J. (2003a). Schistosomiasis control in Ghana: case management and means for diagnosis and treatment within the health system. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 97*(2), 146-152.

128. van der Werf, M. J., & de Vlas, S. J. (2004). Diagnosis of urinary schistosomiasis: a novel approach to compare bladder pathology measured by ultrasound and three methods for

hematuria detection. *The American Journal of Tropical Medicine and Hygiene, 71*(1), 98-106.

129. van der Werf, M. J., de Vlas, S. J., Brooker, S., Looman, C. W. N., Nagelkerke, N. J. D., Habbema, J. D. F., et al. (2003b). Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Tropica, 86*(2-3), 125-139.

130. van der Werf, M. J., de Vlas, S. J., Landouré, A., Bosompem, K. M., & Habbema, J. D. F. (2004). Measuring schistosomiasis case management of the health services in Ghana and Mali. *Tropical Medicine & International Health, 9*(1), 149-157.

131. Vennervald, B. J. a. D., D.W. (14 - 16 November 2005). *Measuring schistosomiasis morbidity.* Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

132. Vlassoff, C., & Tanner, M. (1992). The relevance of rapid assessment to health research and interventions. *Health Policy and Planning, 7*(1), 1-9.

133. Wang, L. D., Chen, H. G., Guo, J. G., Zeng, X. J., Hong, X. L., Xiong, J. J., et al. (2009). A strategy to control transmission of *Schistosoma japonicum* in China. *New England Journal of Medicine, 360*(2), 121-128.

134. Warren, K. S., Siongok, T. K. A., Houser, H. B., Ouma, J. H., & Peters, P. A. (1978). Quantification of infection with *Schistosoma haematobium* in relation to epidemiology and selective population chemotherapy. I. Minimal number of daily egg counts in urine necessary to establish intensity of infection. *The Journal of Infectious Diseases, 138*(6), 849-855.

135. Watts, S. (14 - 16 November 2005). The social determinants of schistosomiasis. Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

136. Wilson, R. A. (14 – 16 November 2005). *Genomics and proteomics: towards new targets in schistosome research.* Paper presented at the Report of the Scientific Working Group on Schistosomiasis, Geneva.

137. World Health Organization. (1993). The control of schistosomiasis: Second report of the WHO Expert Committee, Geneva.

138. World Health Organization. (2000). Report of the WHO informal consultation on schistosomiasis in low transmission areas: control strategies and criteria for elimination, Geneva.

139. World Health Organization. (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis: *Report of a WHO expert committee*, Geneva.

140. World Health Organization. (2003b). Examination of Urine. In *Manual of Basic Techniques for a Health Laboratory* (2 ed.). Geneva.

141. World Health Organization. (2003a). School deworming at a glance. Geneva.

142. World Health Organization (2004). Water, Sanitation and Hygiene Links to Health: Facts and Figures, Geneva.

143. World Health Organization. (14 - 16 November 2005). Report on Schistosomiasis. Paper presented at the Scientific Working Group on Schistosomiasis, Geneva.

144. World Health Organization. (2006). State of the art of new vaccines: research and development, Geneva.