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Paul Livingston Date

Improving Diagnostic Ability and Assessing Prevalence of Schistosomiasis in Lusaka, Zambia

By

Paul Livingston
Master of Public Health

Hubert Department of Global Health

_____

Dr. Susan Allen, MD MPH
Faculty Thesis Advisor

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By

Paul Livingston

Bachelor of Arts, Anthropology
Western Michigan University
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Dr. Susan Allen, MD MPH
Faculty Thesis Advisor

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Health
2012

Abstract

Improving Diagnostic Ability and Assessing Prevalence of Schistosomiasis in Lusaka, Zambia

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Background: Of the 207 million cases of schistosomiasis worldwide, 90% are in Africa, causing hundreds of thousands of deaths annually. Urogenital schistosomiasis (Schistosoma haematobium) has been implicated as a cofactor affecting HIV-1 transmission in many countries of sub-Saharan Africa through the creation of lesions, bleeding and other genital abnormalities. Yet in Zambia, a country experiencing high prevalence of both HIV-1 (13.5%) and schistosomiasis (estimates of 10-40%), few studies have examined prevalence of active schistosomiasis in a population of HIV+ and HIV- urban men and women.

Methods: Participants from the Zambia Emory HIV Research Project heterosexual cohort were included: HIV+ men and women who had tested positive for schistosomiasis antibodies (n=68) and HIV+ and HIV- men and women who came over the period of one month (n=99). Egg excretion was tested utilizing urine filtration and Kato-Katz fecal smear analysis. Plasma samples were analyzed through enzyme linked immunosorbent assays (ELISA) and Western blot for antibodies and to distinguish between schistosome species. Bivariate tests for association were conducted and a multivariate logistic regression model constructed to predict presence of active schistosomiasis infection.

Results: 21% of HIV+ individuals, selected with positive ELISA serologies, had active excretion of schistosome eggs, 19.1% positive for S. haematobium and 7.4 % positive for S. mansoni. In comparison, 13% of unselected HIV+ and HIV- men and women had active excretion of schistosome eggs with 10 % positive for S. haematobium. Logistic regression produced a model predictive of active schistosomiasis infection (AUROC=0.80) that contained variables for age, high ELISA antibody titer (>50), water contact as a child, positive Western blot result for S. haematobium, years in Lusaka and A. lumbricoides positivity.

Discussion: This study shows significant prevalence of active infection in both HIV+ and HIV- Zambians and the need to conduct routine parasitological testing and treatment in countries coendemic for HIV-1 and schistosomiasis. In developing countries, efforts need to be made to provide praziquantel and train laboratory technicians in better diagnostic methods. Future research should examine how male genital schistosomiasis affects HIV-1 transmission and utilize urine reagent strips to test larger numbers of people in high transmission areas of Zambia.

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ACKNOWLEDGEMENTS

Dr. Susan Allen, I am deeply grateful for your kindness, support and constructive criticism throughout the research and writing process. Your continued scholarship and passion in the field of HIV is an inspiration to us all.

Dr. Evan Secor, Molly Hyde and Yeuk-Mui (Pat) Lee, you helped acquaint me with the world of laboratory science and imbued by study with rigor and depth, for which I am profoundly grateful. The Parasitic Disease Branch of the Centers for Disease Control and Prevention graciously gave me space in their freezers, their labs and their hearts by giving me important and insightful criticisms.

Raul Macuacua and Kenneth Ngului, your friendship and support in the field and in the lab at ZEHRP was invaluable and I miss you both deeply.

Dr. William Kilembe, Director of ZEHRP Lusaka, a brilliant and warm man who helped this study run as smoothly as possible and made my experience so enjoyable. Zikomo!

To all the Zambian laboratory technicians, nurse counselors, drivers and general staff, your kindness and assistance helped so much.

To the interns Tyronza Sharkey, Jake Pry, Kahler Stone, Latonya Reinhardt, Cecile Dinh and Ashley Appigyei, your assistance to support the smooth running of this study and the many good times we had in Lusaka will live long in my memory.

To my fellow graduate students Briyana Domjahn and Chad Stegeman, thanks for the long talks, bootleg movies and great friendship in Zambia, and beyond.

To Elizabeth Bradshaw, thank you for your love, understanding and support while I was far away and compassion during the writing of this thesis. I love you with all my heart; you are my soulmate and best friend.

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CHAPTER 1: INTRODUCTION AND BACKGROUND

Schistosomiasis, a parasitic worm disease, is a severe cause of morbidity and mortality in much of the developing world. Of the estimated 207 million worldwide cases, ninety percent are in sub-Saharan Africa (Hotez 2009a) where it causes 300,000 deaths annually, mostly in resource poor countries with limited budgets and staff for health programs. Schistosomiasis is responsible for an estimated 56.6 million DALYs lost annually due to chronic illness and death, more than malaria, tuberculosis and road traffic accidents (Hotez 2007). A complex disease with ecological, spatial, socio-cultural, economic and gender dimensions yet with a simple and inexpensive course of treatment, anti-schistosomal medication still only reaches five percent of those infected across Africa (Hotez 2009b).

In Africa, the common species of schistosomiasis present are the haematobium species which affects the urinary and reproductive tracts and Schistosoma mansoni which affects the hepatic and gastrointestinal systems (Nour 2010). In haematobium endemic areas of sub-Saharan Africa (such as Zambia, in which this research was carried out), urogenital schistosomiasis (UGS) and sexually transmitted infections co-exist, posing a "diagnostic challenge for health care providers" in the management of patients with urogenital complaints (Kjetland 2008b), especially in settings that lack adequate laboratory facilities. This has also led to a growing body of literature on the role schistosomiasis may be playing in Human Immunodeficiency Virus (HIV) transmission in co-endemic countries (Chapter 2). In countries such as Zambia, little is known about the prevalence of schistosomiasis in adults, particularly in populations in which individuals may be in HIV concordant positive or HIV discordant relationships. More data and practical diagnostic tools are needed for health care providers, on the ground in developing countries, to best treat their patients and to understand the link between schistosomiasis infection and HIV transmission.

Traditionally a disease studied in school-age children due to the ability to get large numbers and have an easily accessed, captive population for an intervention study (Michelson 1989), most researchers have conceived of schistosomiasis as a disease affecting rural children which is diagnosed primarily through hematuria (blood in the urine). However, this approach ignores current trends of urbanization sweeping sub-Saharan Africa and the association of urogenital and intestinal schistosomiasis with severe infectious and chronic diseases. In studying the interaction of schistosomiasis and HIV-1 in Zambia, perceptions of what constitute "rural" and "urban" diseases need to be revised. Abundant evidence now shows rural transmission of HIV (Ndhlovu 2007) as well as schistosoma endemicity within urban areas (for Lusaka; Modjarrad 2005, Simoonga 2008). These two epidemics have met where there are migrating populations, commuting spouses, roadside villages and migratory sex work (Ndhlovu 2007), characteristics common in Zambia.

This cross-sectional project aimed to introduce several diagnostic tools (Chapter 3) which could help laboratory technicians and healthcare workers, in a resource-challenged country endemic for both HIV and schistosomiasis, best detect, understand and, if necessary, treat schistosomiasis. Looking at two cohorts: individuals and couples in HIV concordant positive relationships and individuals and partners in HIV discordant relationships, it was hoped that some understanding of the prevalence of schistosomiasis in a population of HIV discordant Zambians could be gained as well as testing whether laboratory measures of parasite antibodies were supported by active infection found in the field. Also, several demographic, water exposure and clinical variables were assessed for statistically significant associations with having active infection for schistosomiasis (as measured in the field).

Recent recommendations by the WHO working group on urogenital schistosomiasis (WHO 2009) have stated that more studies looking at genital schistosomiasis in the context of HIV endemic countries are needed and that treating HIV cofactors may be the most policy-sensitive and least expensive interventions with the most immediate return on investment (Sawers 2008). It is vital to support local health care workers and laboratory technicians in developing countries with the most appropriate tools and information available in order to facilitate fast diagnosis and treatment of the patient, if needed. In the context of schistosomiasis, expensive technologies exist that can diagnose parasitic infection in western laboratories or private clinics in the capitals of affected countries. However, this technology and training rarely extends down to the clinic or community health care worker level for easy dispersion and implementation on a policy level. Knowledge gleaned from studies of helminth infections of HIV infected individuals or HIV discordant relationships can be invaluable in targeting treatment interventions, vector control activities as well as integrating antihelminthic drugs with ongoing HIV prevention and care programs.

Learning what demographic variables and clinical measures are predictive for having active schistosomiasis will better inform policy makers and program managers in Zambia as to what groups should best be targeted, where these groups can best be reached and what form the interventions should take. Comparing laboratory measures using validated and highly specific and sensitive techniques to field-appropriate methods of testing for egg excretion will allow clinic-level staff to have access to low-cost, effective diagnostic tools and to understand their strengths and limitations. An initiative, such as this, which aims to bundle control and treatment of schistosomiasis into a vertical HIV testing and vaccine program, can help to educate local clinic staff on parasite diagnosis, testing and treatment, to be aware of certain characteristic

symptoms in their HIV positive clients and their partners and to bring together disparate Zambian specialists working in health (environmental health, gynecology, parasitology, HIV prevention) in order to craft the most impactful interventions which best treat this parasite in the local context in which it occurs.

DEFINITION OF TERMS/ACRONYMS

AIDS-Acquired Immunodeficiency Syndrome

Bilharzia-The locally understood term used in Zambia for schistosomiasis, coming from Theodor Bilharz, who originally discovered the worm that causes urinary schistosomiasis, in 1851.

ELISA-Enzyme-Linked Immnosorbent Assay, laboratory based test for schistosoma antibodies using plasma samples from study participants

FGS-Female Genital Schistosomiasis, defined as having at least one of the following genital manifestations: sandy yellow patches, grainy sandy patches, contact bleeding and abnormal blood vessels.

GFATM-Global Fund for AIDS, Tuberculosis and Malaria

HIV-Human Immunodeficiency Virus, the virus that causes AIDS

HIV Concordant Positive Relationship-An intimate partnership where both partners are HIV-positive

HIV Discordant Relationship-An intimate partnership where one partner is HIV-positive and the other HIV-negative

MDA-Mass Drug Administration, (in the context of schistosomiasis), of the antihelminthic drug praziquantel.

MGS-Male Genital Schistosomiasis, defined as presence of schistosoma ova in semen or genital pathology.

MOH-Ministry of Health

PEPFAR-President's Emergency Plan for AIDS Relief

WHO-World Health Organization

CHAPTER 2: LITERATURE REVIEW

Several recent meetings and publications have built momentum towards the idea of carrying out studies to examine schistosomiasis within the context of HIV infected and affected populations in Zambia. A critical 2008 workshop on Schistosomiasis and Reproductive Health (Kjetland 2008b) was held in Lusaka, Zambia and brought together the world's leading researchers in order to synthesize the literature on schistosomiasis and reproductive health in sub-Saharan Africa and how these findings could be operationalized in the Zambian context. In 2009, a WHO working group convened in Geneva, Switzerland (WHO 2009) to produce a document that summarized knowledge about male genital schistosomiasis, female genital schistosomiasis, used quantitative studies to build the case for a link between HIV-1 and Schistosoma haematobium and make policy recommendations for routine testing and treatment of at risk populations in endemic countries. Finally, in 2010, a Gates Foundation sponsored Female Genital Schistosomiasis Workshop was held in Copenhagen, Denmark which brought together practitioners from the spheres of HIV prevention and testing, parasitology and obstetrics and gynecology to discuss findings and develop innovative approaches to integrating HIV prevention and schistosomiasis control in Africa.

The Lifecycle of Bilharzia/Snail Fever

Schistosomiasis is a parasitic disease caused by several species of worms of the genus Schistosoma. Snails are natural reservoirs of the parasite, which, in Zambia, consist of the Bulinus globosus species, naturally found in rivers and streams across the country (Simoonga 2008). Humans acquire the disease by coming into contact with bodies of water that have infected snails, mostly through bathing, washing of clothes or swimming. Humans can release eggs into the environment through urinating or defecating in freshwater and thus contaminating

the water source with S. haematobium (urogenital) or S. mansoni (intestinal) eggs. As the eggs hatch in the water, they release miracidia, small larval worms (Cromley 2012). These penetrate the snails and produce a new life stage as sporocysts. The sporocysts divide to produce cercariae, which have the ability to infect humans (Hotez 2009a). Cercariae are released from the snail on a daily basis into the aquatic environment and upon coming into contact with human skin; they attach and penetrate the surface. Migrating to the bloodstream, parasites mature after six to eight weeks and can start producing up to 300 eggs per day (Secor 2005). These eggs then migrate to the portal veins, the intestinal tract (mostly S. mansoni) and the urogenital tract (S. haematobium) and are excreted back out into the environment to begin the lifecycle again.

Schistosomiasis as a Genital Condition

Schistosomiasis was first diagnosed in genital tissue in 1899 in Egypt (Kjetland 2005) and, by 1953, Gelfand and Ross had noted that ova of S. haematobium could be found as a common infection of the female genital tract across Africa (Poggensee 2001). In the mid to late-1990's, important papers from Kjetland (Kjetland 2006), Helling-Giese (1996) and Leutscher (1998) defined Female Genital Schistosomiasis as an important clinical diagnosis for women excreting S. haematobium ova in their urine (Kjetland 1996) in Malawi and hematuria (blood in the urine) and dysuria (painful urination) in Madagascar (Leutscher 1998). Feldmeier (Feldmeier 1999) later identified genital schistosomiasis in men through haemospermia (blood in the ejaculate) by the presence of S. haematobium ova in the seminal fluid and hypothesized a possible "increased shedding of HIV".

The list of genital manifestations attributable to S. haematobium infection has since been broadened to incorporate the growing body of knowledge gathered from medical studies in endemic countries. Though, female genital schistosomiasis can infect any genital organ, it

primarily infects the cervix, followed by the vagina and fallopian tubes (Kjetland 2012), which has important implications for susceptibility to HIV transmission and infection (see below). In a study of 483 adult women in Zimbabwe, Kjetland (Kjetland 2008a) found five clinically visible manifestations of S. haematobium ova in genital tissue; contact bleeding upon vaginal inspection, pre-contact bleeding, both yellow sandy and grainy sandy patches as well as abnormal blood vessels. The WHO working group further (WHO 2009) found that for FGS, symptoms could also include dyspareunia (pain during sexual intercourse), lower abdominal pain, ectopic pregnancy, infertility (as did Schanz 2010 in Nigeria) and "menstrual irregularities" (WHO 2009). In addition to Feldmeier's findings on MGS, the WHO defined the condition as "the presence of ova in the semen or genital pathology" (WHO 2009) but also noted that this included symptoms such as erectile dysfunction, painful ejaculation and infertility.

Diagnosing Schistosomiasis

A variety of methods (depending on the type of sample and resources available) have been employed to accurately diagnose both schistosomiasis positivity and intensity of infection. Morphologically, though of similar size (roughly 115-170μm by 40-70μm), S. haematobium and S. mansoni ova differ in shape, such that under microscopy, S. haematobium can be clearly distinguished by its terminal spine (Montressor 1998) from S. mansoni with its lateral spine. In low-resource settings, urine filtration or urine dipsticks are often used to diagnose and measure intensity of haematobium infection (Gundersen 1996) while Kato-Katz kits using fecal smear analysis are used for measuring intensity of mansoni ova per gram feces (Peters 1980). Details and limitations of these methods (and their suitability for use in Zambia) are discussed in later chapters.

Other laboratory or physician-operated methods hold great promise for offering high sensitivity and specificity but require the appropriate facilities, equipment and trained staff to perform them. The Enzyme-Linked Immunosorbent Assay (Elkawaz 2009, Doenhoff 2004) has been showed to be a reliable laboratory based test to measure the level of schistosomiasis antibodies from plasma and serum samples. The gold standard Western Blot procedure allows researchers to determine not only if the individual has antibodies but what species of schistosomiasis the individual was exposed to. Western Blot has been shown to be highly specific for S. mansoni (Tsang 1983, Maddison 1985) and for S. haematobium (Sulahian 2005) although criticisms have been made of its lack of sensitivity and robustness (Corstjens 2008). Other diagnostic methods such as the usage of a schistosomiasis polymerase chain reaction (PCR) to detect haematobium DNA in urine samples (Mutapi 2011) and the usage of non-invasive ultrasound technology to detect the presence of genital lesions (resulting from S. haematobium infection) (WHO 2009) have been put forward but require more training of practitioners and studies in order their feasibility in endemic countries.

Treating Schistosomiasis

Fortunately, thanks to generic medications and drug donation programs spearheaded by the Gates Foundation, WHO, the United States Agency for International Development and the UK based Department for International Development (Hotez 2009b), anti-schistosomal prophylaxis and treatment are available for eight cents per tablet to endemic countries (Hotez 2009a). The anti-helminthic drug, praziquantel, is a non-toxic drug that is safe for pregnant women and children and has been shown effective at reversing genital pathology (sandy patches) in a study of 21 women with FGS in Malawi (Richter 1996) as well as elimination of urinary ova excretion in a study of 527 women in Zimbabwe (Kjetland 2006a). Praziquantel prevents further

disease and egg deposition through the killing of mature worms in the body of infected individuals (WHO 2009), however, as a prophylactic measure, mass drug administration of school aged children (particularly young girls) shows great promise to prevent future morbidity and mortality. In fact, it has been estimated by Kjetland (Kjetland 2008a) that even a single treatment in childhood could prevent half of the future cases of female genital schistosomiasis.

Finally, praziquantel treatment in individuals co-infected with HIV-1 has been shown to be as effective as in HIV-1 negative individuals (Secor 2006), to be safe and to possibly even attenuate HIV replication by decreasing systemic inflammation (Erikstrup 2008). An extensive review of the literature found few randomized control trials existing that addressed this issue but they seemed to support the findings that antihelminthic drugs may act to reduce viral load and increase CD4 level (Walson 2009). This has important implications for its usage in HIV infected populations and the possible integration of anti-schistosomal chemotherapy in HIV prevention and care programs.

Schistosomiasis in Zambia

Zambia is a country that has a variety of factors (socioeconomic, geographic and developmental) that contribute to the heavy burden of morbidity and mortality experienced by its citizens. In a masterly early synthesis of the literature (Michelson 1989), Michelson traces schistosomiasis in Zambia from the first description of suffering from S. haematobium described by David Livingstone in 1855 down to the present. Zambia is a large country, divided up into eight provinces, that is sparsely populated in most rural areas but has sprawling urban populations and peri-urban slums in the capital Lusaka and the twin cities of the Copperbelt, Kitwe and Ndola.

Water resources play a major role in both rural and urban life as the country has 2250km of inland waterways, extensive areas of marsh and swamp and five major lakes (Michelson 1989), all of which provide habitats for snail hosts. Additionally, positioned just south of the Congo Basin, Zambia has a long and intensive rainy season from October until April, which frequently leads to flooding of rural villages and urban slums and markets. UN figures have shown that only 45 percent of Zambians have access to an improved water source and 55 percent are without access to improved sanitation (Watts 2005), crucial variables that affect schistosomiasis endemicity.

Most studies on prevalence of schistosomiasis in Zambia have only been done in schoolchildren for purposes of convenience already described. In a study of 1583 schoolchildren in peri-urban compounds of Lusaka (Agnew-Blais 2010) 20.7 percent were found to have schistosomiasis infection. The authors noted a "hybridization of urban and rural models of water supply infrastructure" (Agnew-Blais 2010), haphazard urbanization and infrastructure and that few infections were being diagnosed at government clinics. Other studies by Mubila (Mubila 2002) found 76 percent prevalence in schoolchildren on the shores of Lake Kariba and 51 percent in Lusaka schoolchildren, while Simoonga found a much lower prevalence of 9.6 percent in schoolchildren throughout Lusaka province (Simoonga 2008).

This lower prevalence finding can be explained as inclusion of a much larger population size (n=1912) and incorporating areas of Lusaka province with fewer water bodies for schistosomiasis endemicity. More relevant to my study, Schur (Schur 2011) developed an age-heterogeneous model using existing surveys to predict prevalence of haematobium in adults over 20 years at 17.9% and prevalence of mansoni in adults over 20 years at 9.2%. Modjarrad (Modjarrad 2005) explored helminth infections of 297 HIV positive adults in Lusaka and found

that 25 percent had at least one intestinal helminth and that 2.4 percent were positive for S. mansoni infection (haematobium was not measured).

Michelson details that schistosomiasis prevalence may be vastly underestimated in Zambia (particularly in urban areas), noting that at University Teaching Hospital (Lusaka), of 217 cases of bladder cancer (an end-stage manifestation of S. haematobium infection), 65 percent were associated with schistosomiasis (Michelson 1989). National prevalence figures have been difficult to ascertain and estimates vary as widely as 10-40 percent (personal communication, Dr. James Mwansa, Ministry of Health, Zambia). Understanding urban transmission of schistosomiasis in Zambia as well as helminth prevalence data for HIV infected adults requires more studies, particularly longitudinal studies able to follow individuals and couples over time to observe morbidity, mortality and seroconversion (Modjarrad 2005b, WHO 2009).

HIV-1 and Schistosomiasis

Urogenital schistosomiasis and sexually transmitted infections co-exist in most Schistosoma haematobium endemic areas of sub-Saharan Africa (Leutscher 1998) and much has been made of the extensive geographical overlap of endemic HIV-1 and schistosomiasis areas of Africa (Hotez 2009a, Noblick 2011). Circumstantial evidence for the relationship between urogenital schistosomiasis and the transmission of HIV-1 was first described by Feldmeier (Feldmeier 1994) for lesions of the female genital tract caused by S. haematobium infection. A compilation of studies reviewed by Poggensee (Poggensee 1999) for Niger, Malawi, Madagascar and Tanzania built upon Feldmeier's theory by first describing the biological mechanisms by which both S. haematobium and S. mansoni might increase a woman's risk of acquisition and transmission of HIV-1. This paper (along with Poggensee 2001) described the action of

urogenital schistosomiasis to create lesions in the epithelium of the genital tract and to initiate a protective immune response. As previously described, the primary site of S. haematobium ova deposition is on the cervix, which research has shown to also be the prime site of HIV transmission (Kjetland 2012).

For women with urogenital schistosomiasis, infection may have occurred as a young girl, affecting the barrier function of the vulva and vagina and impairing the cervical epithelium before first sexual intercourse (WHO 2009). In endemic countries of sub-Saharan Africa, sexual relationships are frequently initiated shortly after menarche (Poggensee 2001), making the vulnerability of S. haematobium young girls to HIV particularly acute. The presence of female genital schistosomiasis primarily acts by causing breaks and lesions in the vaginal and cervical epithelium and initiating an immune response which facilitates easier HIV transmission. The inflammation, erosion and ulceration (Kjetland 2006) manifested in women with genital schistosomiasis act in a similar fashion as sexually transmitted infections, causing breaches in the mucosal epithelium for HIV-1 to enter or leave. Furthermore, neovascularization (abnormal blood vessels) of the epithelium allow direct access of HIV-1 to the systemic circulation of the receptive partner (WHO 2009). The immune response recruits lymphocytes, macrophages and Lagerhans giant cells (WHO 2009), expressing CD4 cells at the site of inflammatory lesions (Secor 2006) and adding to the probability of HIV infection. Noblick has also described the "immunomodulatory" effect of helminth infections and how they act to diminish a host's immunity to HIV by promoting viral replication and T cell diminution (Noblick 2011). Thus, relatively inefficient mucosal transmission of HIV-1 is made much more efficient through inflammatory lesions and the presence of abundant CD4 cells for HIV-1 to attach to (Poggensee 2001).

This immunomodulatory effects on HIV susceptibility have been studied more extensively for S. mansoni (Mbabazi 2011) than S. haematobium. In a study of male car washers in Kenya who were co-infected with HIV-1 and S. mansoni, Secor found that certain receptors (CCR5 and CXCR4) which HIV uses for binding were denser on CD4 T-cell surfaces of individuals actively infected with S. mansoni compared to those on CD4 T-cells of individuals who had been treated with praziquantel (Secor 2003). In primate studies, Sidappa et. al. found that rhesus macaques infected with S. mansoni were more likely to develop systemic HIV infection (after rectal HIV exposure) than macaques without S. mansoni infection (Sidappa 2011), likely due to "mucosal inflammation" as a result of ova deposition. Secor further found that HIV positive patients with S. mansoni could be as effectively cured of infection as HIV negative patients (Secor 2006), showing that in co-infected patients, praziquantel retains its efficacy to clear schistosomal infection.

A seminal study that showed a quantitative link between genital schistosomiasis and HIV-1 acquisition was Kjetland's cross-sectional study of 527 women in Zimbabwe. She found that women with urogenital schistosomiasis had a threefold risk of having HIV as women without urogenital schistosomiasis, after a one year follow-up (Kjetland 2006b). In addition, S. haematobium infection of the genital mucosa was found to be statistically associated with HIV seropositivity and, of the seven women who became HIV positive during follow-up, all seven had signs of S. haematobium at baseline (Kjetland 2006b). Another cross-sectional study done in Zimbabwe by Ndhlovu on 544 women aged 15 to 49 years found women with urogenital schistosomiasis to have an HIV prevalence of 33 percent while women without UGS to have an HIV prevalence of 25.6 percent ($p=0.053$) (Ndhlovu 2007). But due to the paucity of quantitative studies, the lack of long-term longitudinal studies to follow individuals for seroconversion

(Mbabazi 2011), the neglect of men and male genital schistosomiasis and the difficulty of studying genital schistosomiasis in endemic countries with their attendant logistical and cultural challenges (Kjetland 2012), the link between UGS and HIV-1 remains tenuous and largely theoretical.

However, researchers from other disparate fields such as gynecology, mathematics and economics have recognized the importance of this issue of linkage between a parasitic infection and HIV-1 and produced research to buttress public health's growing body of literature that such a linkage should inform policy and intervention practice. Nawal Nour (an OBGYN) at Harvard has shown that not only do schistosoma ova-induced lesions appear in the mucosa of the cervix and vagina but also that external vulvar or perianal lesions may develop in up to 30 percent of women with FGS (Nour 2010) and that praziquantel treatment to prevent lesions can lead to decreased HIV-1 transmission. In the absence of concrete longitudinal studies showing linkages between UGS and HIV, mathematical models have been developed by Bhunu and others that have shown that infection with schistosomiasis enhances the risk of HIV infection per sexual contact (Bhunu 2010) through egg-induced injury to the reproductive organs. In a multi-disciplinary literature review of the evidence for UGS-HIV1 linkage as well as the cost-benefit effectiveness of various health interventions, economists Sawers and Stillwaggon identify schistosomiasis as an important cofactor of HIV transmission and make the case that treating schistosomiasis is a cost-effective add-on to HIV prevention and treatment programs, capable of slowing the spread of HIV in disease-burdened populations (Sawers 2008).

Poverty and Socio-determinants of Infection

The associations between poverty, poor sanitation and infection with schistosomiasis have been demonstrated across Africa and bear important consideration in planning upstream

prevention activities (Nour 2010). Watts, in her literature review on the social determinants of schistosomiasis infection found that the face of schistosomiasis was disproportionately poor and female as women were found to have more regular water contact (washing, carrying household water) than men (Watts 2005). She also examined the role that rural to urban migration, dams (see section below) and urbanization had on schistosomiasis transmission and found rural to urban migration to be responsible for initial exposure of non-immune urban populations to a once rural disease and the beginnings of endemic transmission within urban areas of Africa (Watts 2005).

In a study of 1023 young people (aged 9-25) in southwestern Nigeria, Ugbomoiko the direct link between poverty and infection with schistosomiasis, finding a protective role for literacy, higher education and improved housing (all correlates of elevated socioeconomic status) (Ugbomoiko 2010). Compiling evidence based studies from Zimbabwe (Kjetland 2006b) and Madagascar (Leutscher 1998), Mbabazi termed schistosomiasis "a disease of poverty that arises in areas of poor sanitation" (Mbabazi 2011) as people come into contact with water contaminated with human waste as part of their daily lives. However, more studies are needed to examine the role of poverty in urban and peri-urban schistosomiasis transmission and not simply in rural populations.

The Role of Dams

The destructive role played by dams and large hydrological projects in creating areas of snail habitat and increasing schistosomiasis transmission has been described in a historical, global context (Jobin 1999), in China (Li 2007) and for Zambia in particular (Scudder 2005, Steinmann 2006). Jobin described how the creation of dam canals was able to harbor Bulinus snails and how the reduced flow of the water and the creation of dammed lakes created ideal

snail habitats and riverbank ecology conducive to snail infestation. In a study of dams in the Dongting Lake region in China, Li (Li 2007) showed that dams substantially extended the range of snail habitats and increased number of schistosomiasis cases. Li also conducted a meta-analysis of data on African hydrological projects and calculated risk ratios of 2.4 for S. haematobium and 2.6 for S. mansoni for persons living adjacent to dam reservoirs compared to those living farther away (Li 2007).

In Zambia, much of the studies have looked at the construction of Kariba Dam and the subsequent damming of the Zambezi River in 1958. This massive damming project created Lake Kariba (with 2164 kilometers of shoreline) and created extensive shoreline with protected coves for the Bulinus globosus snail to propagate (Jobin 1999). At the time of construction, there was low prevalence of S. haematobium in the area (southern province of Zambia, near the border with Zimbabwe), no endemic S. mansoni transmission and no snail intermediate host population (Steinmann 2006). Only eighty years later, prevalence of S. haematobium, in villagers living on the banks of the newly created Lake Kariba, was found to be 29 percent and 6 percent for S. mansoni. In the 1990s, these figures increased to a prevalence of 35 percent for S. haematobium and 45 percent for S. mansoni around Lake Kariba (Scudder 2005, Steinmann 2006). In a study of 684 schoolchildren around Lake Kariba and in areas around Lusaka, Mubila found prevalence of schistosomiasis in schoolchildren around Lake Kariba at 76 percent and 51 percent for Lusaka schoolchildren (Mubila 2002). Two sites of ecological disruption affecting schistosomiasis prevalence were identified for the two sites, the Kariba Dam and Lake Kariba for southern province and Chasinama Dam outside Lusaka (Mubila 2002).

Criticisms of the Literature

The gaps in the broad swath of scholarship on the background of schistosomiasis, how best to prevent it, how best to diagnose it, how best to treat it and the parasite's role as a possible cofactor in HIV transmission in co-endemic countries show just how much more research is still needed. Most studies have focused on children (Michelson 1989), considering schistosomiasis a disease of rural children while ignoring the dynamic nature of urbanization and rural to urban migration in Africa. Similarly problematic is the approach of many of those in the field who proscribe praziquantel mass drug administration (MDA) as the cure to schistosomiasis transmission as well as a way to slow vertical and horizontal HIV transmission (Fenwick 2006, Hotez 2009a, WHO 2009). This mass treatment plan is overly simplistic and ignores environmental strategies such as water treatment, improvement in water access and sanitation and application of molluscicides to kill snails would reduce transmission and prevent future re-infection.

In relation to the theorized link between HIV-1 and Schistosomiasis infection, few quantitative studies have been able to show a statistically significant linkage and demonstrate a clear cause-effect relationship (apart from Kjetland 2006b). The short-term nature of most cross-sectional studies with little follow-up has meant that individuals and couples are not able to be followed longitudinally for seroconversion and other clinically important measures which may better explain the role that S. haematobium and S. mansoni play in HIV-1 acquisition and transmission. Secondly, the vast majority of studies examining genital schistosomiasis have been done in women with little attention paid to abnormal genital pathology in men caused by S. haematobium or its presence in urine or semen (apart from the work of Leutscher (Leutscher 1998)). More studies testing for genital lesions in men, for ova excretion in urine and testing

semen for schistosomiasis and HIV viral load are necessary to better understand the role that MGS may be playing in men infected and co-infected with schistosomiasis and HIV-1.

Finally, too many studies using clinical or laboratory measures in the diagnosis and treatment of schistosomiasis ignores the real-life resource constraints of local health workers in endemic countries such as Zambia. The usage of schistosomiasis PCR (Mutapi 2011), ELISA (Elkawaz 2009) and Western Blot (Sulahian 2005) testing for antibodies and utilization of ultrasound (Helling-Giese 1996) and colposcopy (WHO 2009) have all been shown to be efficacious in diagnosing and recognizing infection with schistosomiasis. But expensive testing in well-funded laboratories in the capitals of endemic countries or back in Europe or the United States without training local laboratory technicians or providing appropriate laboratory facilities in developing countries is unsustainable and unethical. More emphasis should be placed on making available cheap and easily understood diagnostic tools to laboratory technicians, OBGYNs and doctors in countries such as Zambia and on training these health care workers in the utility and usage of new diagnostic methods (such as colposcopy linked to computerized digital imaging and the new reagent assay validated in Tanzania with a specificity of 90 percent and requiring little technical training or equipment (Van Dam 2004)).

CHAPTER 3: METHODS

The cross-sectional study undertaken from June until August 2011 in Lusaka, Zambia aimed to understand the prevalence of active schistosomiasis in two cohorts (individuals and couples in HIV concordant positive relationships and individuals and couples in HIV discordant relationships) as well as the ability of clinical and demographic variables to predict an individual's active schistosomiasis status. In order to measure these variables in both a laboratory and clinic setting, certain techniques were utilized for their non-invasiveness, applicability in resource-poor constraints and specificity and sensitivity. The strengths and limitations of the respective clinical and laboratory techniques are discussed below.

Study Background

Lusaka is the capital of Zambia and a growing metropolis consisting of a central urban business and market district surrounded by peri-urban slums and a population of roughly 1.7 million people (Agnew-Blais 2010). The organization, under which this study was carried out, the Rwanda Zambia HIV Research Group (RZHRG) based at Emory University, has operated in Zambia under the title Zambian Emory HIV Research Project (ZEHRP) since 1994. Primarily involved with couples-based voluntary HIV counseling and testing (CVCT) and utilizing partner-based interventions as an "entry-point" to HIV clinical care, ZEHRP has been following cohorts of heterosexual HIV concordant positive and HIV discordant couples longitudinally in order to carry out observational studies, track seroconversion and treat emergent or recurring sexually transmitted infections (STIs) and opportunistic infections (OIs).

These heterosexual cohorts have been described previously for seroconversion rates over time (Dunkle 2008), incident sexually transmitted infections (Reilly 2008) and the relationship between genital abnormalities and presence of antibodies to schistosomiasis (Dinh 2011). Dunkle

et. al. have shown that seroconversion rates within HIV discordant couples who have been jointly tested and counseled ranges from three to seven percent per year, much lower than the 20 to 25 percent per year in couples who have not been jointly tested and counseled (Dunkle 2008). In a representative study of 778 men and women, this cohort has also been shown to have high incidence in STIs, finding 78.5 percent to be positive for herpes simplex virus as well as 35.9 percent presenting with a genital ulcer at follow-up with 50 percent of HIV-positive men having a genital ulcer (Reilly 2008). Directly relating to schistosomiasis, Dinh utilized data for 2168 individuals in ZEHRP's heterosexual cohort, testing for associations between genital abnormalities and presence of antibodies to schistosomiasis (assessed in laboratories at the Centers for Disease Control and Prevention (CDC), Atlanta, GA). Dinh found no statistical association between baseline HIV status or transmission of HIV and antibodies to schistosomiasis (Dinh 2011). However, statistically significant associations were found between some genital abnormalities such as cervical inflammation and gonorrhea and antibodies to schistosomiasis.

Sample Population

The selection of the final study population of two cohorts (individuals and couples in HIV concordant positive relationships and individuals and couples in HIV discordant relationships) is described in Figure 1. For the cohort of HIV positive in concordant relationships (Cohort 1), schistosomiasis antibody titers were known and used as inclusive or exclusive factors to participate in this study. For the cohort of individuals and couples in HIV discordant relationships (Cohort 2), schistosomiasis antibody titers were not known and were tested over a one month period to gather a representative sample of schistosomiasis prevalence in the broader HIV discordant population.

To compile a reduced cohort of HIV positive individuals in HIV concordant positive relationships and drawing on Dinh's study population of 2,168 individuals, exclusions were made for having enzyme-linked immunosorbent assay titers of less than 25 (defined as schistosomiasis negative, see below) and limiting for those individuals still alive and being followed up at quarterly visits. This resultant number of 95 individuals was further reduced in country as 27 additional individuals were excluded due to death, refusal to participate or moving out of the area. The final number of individuals in cohort 1 was 68, of whom there were 11 couples and 46 individuals (both partners were not represented), also of the 68, 40 were men and 28 women. For cohort 2, over a one month period (July 2011), 140 individuals were assessed for study eligibility and 41 excluded due to refusal to participation or inability to provide laboratory samples. Resultantly, in cohort 2, 99 individuals were tested for active schistosomiasis infection, of whom; there were 18 couples and 63 individuals and 51 were men and 48 women.

Research Design

As a cross-sectional study, prevalence and biochemical measures were assessed both in the field (through utilization of syringe urine filtration and Kato-Katz fecal smear analysis) and back in the United States (using ELISA and Western Blot tests). Statistical computation and analysis were undertaken to assess whether prevalence of active schistosomiasis infection differed between the two cohorts and what demographic, clinical or personal variables (arising from usage of a questionnaire) were predictive of having active schistosomiasis infection.

Procedures

Approval for this study was granted by the Emory University Institutional Review Board under the following pre-approved protocols; IRB #356-2004 Heterosexual Transmission of HIV in Africa, IRB #357-2004 Formative Focus Groups and Interviews in Two African Capitals and

IRB #358-2004 One Day VCT Screening Services in Two African Capitals. Ethics approval was also granted in-country by the University of Zambia Research Ethics Committee (Assurance No. FWA00000338). Informed consent was given through hearing a message about bilharzia (locally understood term for schistosomiasis) from nurse counselors (Appendix 2) and giving oral consent. Prior to enrollment into this study, a comprehensive site-specific protocol (Appendix 1) was developed for training of local staff and to make explicit the roles and responsibilities of both the researcher and laboratory technicians as well as explaining mechanisms of quality control.

To test for the presence of schistosome ova in urine, a single midday sample was collected from study participants. The urine samples were collected between the hours of 10am and 2pm, as excretion of S. haematobium eggs has been shown to follow a circadian rhythm with a peak around the noon hours (Montressor 1998). The syringe urine filtration technique of detecting S. haematobium ova in urine has been shown to be effective by Gyorkos (Gyorkos 2001) and by the WHO for measuring severity of schistosomiasis infection (WHO 2008). A Swinnex® (made by Pall Corporation) 47 millimeter filter holder was used together with Fischer Scientific© GE Magna nylon membrane filters of 10.0µm pore size and 13millimeter diameter. Filter holders were reused after thorough washing and drying and filters were used once and discarded in biohazard containers.

To perform syringe urine filtration, the collected urine sample was examined as soon as possible after collection to prevent hatching of eggs or formation of crystals, which could lead to a misdiagnosis (WHO 2008). A sterile 10 milliliter syringe was unwrapped and attached to the filter holder unit containing the sterile 10µm pore filter (see Appendix 1). The urine sample was shaken and mixed and then a 10ml sample drawn up into the syringe. The depressing of the

plunger forced urine through the filter and into a bucket and caught any eggs present in the fine mesh of the filter. The filter was then removed and placed onto a microscope slide, onto which was applied a drop of Lugol's iodine as a stain to enhance the appearance of any resultant eggs, and after waiting thirty seconds, the entire stained filter was examined thoroughly under a microscope at low power (40x). The laboratory where these tests were carried out in Lusaka, Zambia did not have the capability to take digital pictures of slides under microscopy.

Presence of a single terminal-spined egg was diagnostic of infection with Schistosoma haematobium. Intensity of infection was measured in number of eggs per 10 milliliter urine sample and quantified as either a light intensity infection (<50 eggs/10ml) or a heavy intensity infection (>50 eggs/10ml or visible hematuria) (Montressor 1998). In order to ensure quality control of diagnosis, every positive diagnosis by the researcher of ova under microscopy was verified by the attendant senior laboratory technician who had many years of experience utilizing microscopy in the diagnosis of parasitic infections.

The utility of the Kato-Katz kit for fecal smear analysis in diagnosing S. mansoni (and possibly S. haematobium) in stool has been repeatedly shown in resource limited countries (Peters 1980, Montressor 1998, Doenhoff 2004). The Kato-Katz fecal smear kits (made by Vestergaard Frandsen® Europe) contain nylon screen, rolls of cellophane and spatulas. The night before examining stool samples, strips of cellophane were soaked in a methylene blue glycerol solution and the following day applied on to the feces sample to make present eggs more visible. Slides (with fecal smears covered by methylene glycerol soaked cellophane strips) were then left to sit at room temperature for one hour in order to clear fecal material before microscopic examination (WHO 2008). Slides were examined thoroughly and the resultant number of eggs multiplied by a factor of twenty four to arrive at the number of eggs per gram of feces (epg).

Presence of a single egg with lateral spine was considered diagnostic of S. mansoni infection. The intensity of infection (epg) was classified as light infection (1-99epg), moderate infection (100-399 epg) and heavy infection (>400 epg). As with urine filtration, each positive diagnosis was verified by the senior laboratory technician on staff (and if he was not available, verification was made by another laboratory technician).

In addition to testing urine and fecal samples for the presence of schistosoma eggs, the local doctors and nurse counselors requested testing for Ascaris lumbricoides as well. This common parasitic worm can be easily identified in fecal smear analysis under microscopy and treated using the antihelminthic drugs Albendazole and Mebendazole. A. lumbricoides eggs have a characteristic rounded shape and thick shell, differentiating them from the oblong terminal-spined egg of S. haematobium and the oblong lateral-spined egg of S. mansoni. Though not of direct relevance to the findings on schistosomiasis or a possible link to HIV infection, it was considered important to understand the prevalence of polyparasitism in the ZEHRP cohorts of HIV positive and HIV discordant couples.

Treatment

The anti-schistosomal agent Praziquantel was procured locally and given to all individuals found to be positive for S. haematobium and/or S. mansoni as therapeutic chemotherapy. Praziquantel acts to kill all adult worms present in the body and thus prevent further deposition and excretion of eggs. However, lesions, bleeding and other manifestations of urogenital schistosomiasis are not cured and must be treated separately (see Limitations). Praziquantel has been shown to be safe for children, adults and pregnant women (Secor 2005, Kjetland 2012) and was given at WHO-approved dosage recommendations of 40 milligram per kilogram body weight. Praziquantel has no reported issues with drug resistance and few side

effects including headache, dizziness and nausea (Hotez 2009b). In addition, praziquantel is one of the most cost-effective treatments available, clearing adult worms from the body for around 40 cents US per treatment (Secor 2005, Hotez 2009a).

Antibody Testing

In order to test for presence of schistosomiasis antibodies back in the United States, a plasma sample was obtained from every study participant and aliquoted down to a 50µl tube for shipping. Aliquotted specimens were stored in a freezer at ZEHRP laboratories in Lusaka at -80 degrees Celsius from the time of collection until shipping to the United States (approximately three months). Plasma samples were tested for the presence and intensity of antibody titer through enzyme linked immnosorbent assays (ELISA) and verified using Western blot testing at the Centers for Disease Control and Prevention laboratories of Dr. Evan Secor in Atlanta, Georgia. Western blot also allowed the researcher to distinguish between antibodies to S. haematobium and S. mansoni and thus served as a confirmatory "gold standard" for the ELISA tests.

ELISA tests have been shown in the literature to be highly specific and robust laboratory measures of antibodies to schistosomiasis infection (Doenhoff 2004, Elkawaz 2009, Corstjens 2008). ELISA tests were performed on banked plasma samples of study participants using soluble worm antigen preparation (SWAP) and horseradish peroxidase anti-human antibody. Positive antibody titers were determined by calculating three times the standard deviation of the antibody levels of the negative controls (marking low positive titer at 26.34 and high positive titer at 52.68). This was very similar to the levels used by Dinh (25 and above for low positive, 50 and above for high positive) (Dinh 2011).

The usage of the Western immunoblot as a "gold standard" antibody test with the ability to detect schistosomiasis species cross-reactivity has been shown for S. mansoni (Maddison 1985, Tsang 1984) and S. haematobium (Sulahian 2005). Antibody reactivity for 108 sera samples were tested using the Sm25 and Sm29 microsomal antigens of S. mansoni as well as S. haematobium antigen. Examples of positive strip results are shown in Figures 2 and 3.

Questionnaires

Questionnaires have been shown in the literature to be effective tools at assessing history of exposure to schistosomiasis and possible symptoms. They are especially useful in areas where low levels of biomedical technology are available and can be easily used by health workers and community health volunteers (Lengeler 2002a). This has been shown for schoolchildren in Zambia (Lengeler 2002b), who showed, in a study of 7875 children, that questionnaires had 71 percent sensitivity and 73 percent specificity for predicting active infection. Questionnaires have also been used to great effect in Ivory Coast (Utzinger 2000), Ghana (Danso-Appiah 2010) and Nigeria (Ukwandu 2004).

At the time of recruitment into this study and collection of urine, feces and a blood sample, a questionnaire was also administered by the nurse counselor asking demographic questions, questions about history of water exposure and presence of genital symptoms which might be predictive of urogenital schistosomiasis infection (Figure 4). In order to ascertain which areas of schistosomiasis endemicity or bodies of water might lead to exposure, study participants were asked about province and city in which they were born and area in which they currently live. Gender was asked about as women have been found to have disproportionate exposure and morbidity from urogenital schistosomiasis (Watts 2005). Current water usage and source as well as possible childhood water exposure routes were asked about, with the nurse counselors asking

the person to name a specific river, lake or water source to ensure validity of reported information. Gender-specific presence of conditions that are symptomatic of urogenital schistosomiasis were also asked about, while infertility, presence of bloody urine and sexually transmitted infection within the past three months were asked of everyone. Relevant information on genital abnormalities already being tracked by RZRHG were collected and analyzed, including vaginal discharge, urethral discharge, syphilis, gonorrhea, inguinal adenopathy, etc.

Data Analysis

The statistical analyses were computed using Statistical Analysis System (SAS) version 9.3 (SAS Institute Inc., Cary, NC). Descriptive statistics of continuous variables (Table 1) and categorical variables (Table 2) were first calculated for both the cohort of HIV positive individuals and couples in follow-up (n=68) and HIV discordant couples and individuals (n=99). Continuous variables across individuals positive (n=26) and negative (n=136) for active schistosomiasis infection were also calculated (Table 3). ELISA and immunoblot results for patients with and without active schistosomiasis were compiled to show relationships between active infection and laboratory-based antibody measures (Table 4). Bivariate tables were then created for variables thought to be predictive of active schistosomiasis and negative schistosomiasis (Table 5) as well as for individuals HIV positive (n=107) and HIV negative (n=60) (Table 6). Number of missing values for these variables were noted and chi-square p-values calculated, with Fisher's exact two-sided p-values calculated where cell values were values of five or less. For HIV serostatus, descriptive statistics were calculated (Table 7) as well as stratification of schistosome infection by HIV serostatus (for HIV-discordant couples) (Table 8). Gender and its relationship to schistosomiasis and other associated variables are shown in Tables 9-11. Univariate analysis of categorical variables predictive of active schistosomiasis

infections was conducted and crude odds ratios, confidence intervals and p-values calculated (Table 13).

To model the ability of predictor variables to predict the binary outcome (Presence or Absence of Active Schistosomiasis infection), a logistic regression model was constructed. Logistic regression analysis using backward elimination and was applied with a five percent significance level and variables were excluded if the p-value from the crude association was less than 0.2. Variables were also included in the final parsimonious model if they were found to be significant in univariate analysis or had clinical significance in the literature as predictive of having active schistosomiasis ova excretion. Tests of interaction were conducted and interaction terms were not included if p-values were greater than 0.05. Tests of multicollinearity were run and presence of multicollinearity was considered to be indicated if VIF values were greater than three. Area Under the Receiver Operator Curve (AUROC), Likelihood ratio, r-square value, residuals and the Hosmer-Lemeshow Goodness of Fit p-value were calculated and used as indicators of overall model fitness.

Limitations and Delimitations

There were several limitations implicit in both the methodology and diagnostic techniques that undoubtedly affected study results and may have contributed to underreporting or were significant sources of error. A concentrated effort was made by the researcher to design a study that could utilize diagnostic techniques appropriate to resource-limited settings, to train and work cooperatively with local staff and laboratory technicians and make treatment available to those found to be positive with schistosomiasis. However, it is probable that at each step of the diagnostic and treatment pathway, more could have been done given greater time and monetary

resources and that more advanced diagnostic techniques would be able to catch those positive individuals missed in this study (Chapter 4).

For the usage of syringe urine filtration, numerous researchers have criticized the usage of hematuria in urine as diagnostic of urogenital schistosomiasis (Gundersen 1996) as well as the likely underreporting and missing of light infections through collection of only a single urine sample (Kjetland 1996, Poggensee 2000b, Mutapi 2011). Gundersen (Gundersen 1996) has shown that, for women, urine samples may be contaminated with menstrual blood and vaginal secretions which may give false positive readings for hematuria and obscure ova present in the urine. Urine samples should be ideally collected and tested over three days (Poggensee 2000a) and participants given a 300 milliliter drink prior to urine collection (Poggensee 2000b).

Financial constraints associated with the need to reimburse study participants the equivalent of five to ten US dollars (depending on if being called back to give samples or if captured at an already scheduled appointment) and a limited budget and time in country prevented multiple urine samples from multiple days being collected. Kjetland (Kjetland 2012) has termed urine filtration an "insensitive" indicator for diagnosis of genital S. haematobium and though the demonstration of ova in urine confirms the presence of adult worms, the failure to detect eggs does not necessarily exclude active infection (WHO 2009). Furthermore, though measures of quality control were attempted (through training staff on specimen collection and chain of custody transport to the laboratory as well as utilizing a senior laboratory technician to verify the positive diagnosis of schistosomiasis infection by the researcher under microscopy), egg count for each positive urine sample was not verified by a senior laboratory technician and thus has the potential to be undercounted or incorrect.

The Kato-Katz fecal smear analysis technique, though generally more specific and sensitive than urine filtration, is not without its limitations and costs. Speich (Speich 2010) has shown that routine testing of fecal samples in a resource limited laboratory exerts significant temporal and financial costs on already stretched laboratory staff. Though the researcher carried out the Kato-Katz (KK) tests in this study, Speich showed that performing a single KK test costs roughly $1.73 and takes about twenty minutes while performing duplicate KK tests costs $2.06 and takes roughly twenty seven minutes (Speich 2010). For this study, and according to these calculations, laboratory staff would have spent two hundred eighty nine dollars and fifty six hours to perform tests on all 167 study subjects, time and money that might simply not be available. As noted by Speich, KK tests should ideally be performed in duplicate (which was not able to be done in this study) and as with urine samples, fecal samples should ideally be collected on three separate occasions to minimize inter-day variation (Doenhoff 2004). Finally, as for urine filtration, though the researcher had each positive KK test verified by a senior laboratory technician each egg per gram (epg) count could not be verified by senior laboratory staff and thus may represent an undercount or incorrect number.

The questionnaires were useful at identifying demographic variables and prior schistosomiasis exposure but have mostly been used in children with more recent schistosomiasis exposure. Using questionnaires in adults asks them to both remember exposures from perhaps decades ago (thus making this study vulnerable to recall bias) and asks study participants to reveal genital symptoms of illness to nurse counselors (leading to underreporting as a result of social desirability bias). This may be particularly acute when female study participants were answering sensitive questions from male nurse counselors and vice versa. Women in southern Africa have been shown to be reluctant to talk about bloody urine or vaginal discharge

(Poggensee 2000a) thinking they have an illness, which was shown to lead to biased and underreporting of symptoms, as may have happened in this study. The variable, spot bleeding, in particular, may have been underreported on the questionnaire as many women in Zambia do not wear underwear and thus blood spotting is not readily visible (Kjetland 2008b).

For laboratory tests conducted back in the US at laboratories at the CDC, limitations affected the ability of ELISA and Western blot tests to serve as "gold standard" support to findings of active schistosomiasis in the field. ELISA has been shown to have variable sensitivity and specificity measures to detect antibodies to adult worm antigen in laboratory trials (Elkawaz (Elkawaz 2009) showed a sensitivity of 82 percent and specificity of 95 percent while Doenhoff (Doenhoff 2004) found sensitivity of 91 percent and specificity of 90 percent). ELISA tests have been criticized for lacking sensitivity and robustness (Corstjens 2008) meaning that some actual antibody positive samples could have been missed in the laboratory procedures. The usage of antibody data is problematic, as well, because antibody detection cannot "reliably differentiate between past and present infection and antibody correlation does not reliably correlate with number of worms present" (WHO 2009). Lastly, the designation of "low positive titer" and "high positive titer" was relatively arbitrary as it was based on sera from negative controls taken from the United States and not Zambia or even Africa, due to lack of schistosomiasis negative sera from endemic countries.

For the Western blot tests, a significant number of missing data resulting from lack of available sera or tests not previously performed on study participants influenced the ability to directly correlate Western blot results with field results for active infection. Of 167 individuals, 47 sera samples were not present or not able to be tested (28 percent) and, of active schistosomiasis positives, six out of 26 (23 percent) were missing. This missing data may also

have affected the statistical analysis and modeling of the predictive ability of a positive Western blot result to predict a positive active infection, skewing the association into non-significance. Sulahian (Sulahian 2005) also noted the high number of false positives for S. haematobium in Western blot tests and need for specialized research laboratories as making the tests difficult to carry out in endemic developing countries.

Praziquantel, as previously described, is extremely effective at killing adult worms, cost-effective and safe for use in children and pregnant women. However, although praziquantel halts new ova excretion, parasitological cure does not cure genital lesions or resolved contact bleeding, blood vessel abnormalities or other genital manifestations of S. haematobium infection (Hotez 2009a, Kjetland 2006a, Mwanakasale 2003). Antihelminthic treatment is also limited in its effectiveness if study participants are returning to contaminated environments and becoming re-infected with schistosomiasis through water contact, though for immune compromised adults in urban Zambia, this is unlikely.

More broadly, the inability of the researcher or local colleagues (OBGYNs, doctors) to perform detailed female genital exams or analyze male semen samples for schistosomiasis made concrete diagnosis of genital schistosomiasis impossible. As Kjetland has noted, studying genital schistosomiasis in endemic countries is a "huge challenge, both logistically and culturally, requiring lots of funding, basics such as water and electricity, a colposcope and extensive training of staff" (Kjetland 2012). The aforementioned colposcope is a medical device which enables a trained OBGYN to visually inspect the cervix, vagina and vulva with an illuminated and magnified view and capture images with an attached digital camera or laptop. This study did not have usage of a colposcope and in a study of couples or individuals in intimate partnerships; permission from husbands or male partners for a male OBGYN to perform an

invasive colposcopy procedure may have been difficult. Finally, it was not possible to perform two of the most accurate diagnostic procedures; schistosomiasis polymerase chain reaction (PCR) without sophisticated and expensive laboratory infrastructure and cervical biopsy due to ethical concerns about creating an open wound in the genital tract in a population of HIV positive or HIV serodiscordant couples.

Due to the cross-sectional nature of this study, it was impossible to associate positive schistosomiasis with seroconversion in this population. For these HIV-counseled and tested cohorts, an estimated three to seven percent seroconvert per year (Dunkle 2008), which was not able to be captured within the narrow three month window of field work carried out for this study. The WHO has stated that the desired "ideal" study which followed HIV-negative young women with genital schistosomiasis for seroconversion would take an estimated six to eight years (WHO 2009). It was also not possible to determine a cause and effect relationship between HIV infection and schistosomiasis in this cross-sectional study, although rural women are likely to have already had genital schistosomiasis at the time of HIV transmission (Kjetland 2006b).

The study methodology also limited direct comparisons and measures of effect to be understood. The two cohorts used a mixture of couples and individuals, when ideally all HIV positive individuals with high schistosomiasis antibodies should have been represented as individuals and all individuals in the discordant group represented as couples. Linkages between schistosomiasis and HIV status could have been strengthened by including a control group who were HIV-negative or were HIV-concordant negative partnerships as a means to controlling antibody titers between HIV-negative and HIV-positive individuals and couples. The researcher necessarily limited the scope of the study, due to the three month period, to assess prevalence of

urogenital and intestinal schistosomiasis and examine risk factors predictive of having a positive result for active schistosomiasis infection.

RESULTS

For individuals in HIV-concordant positive relationships (n=68: n=35 for ELISA low positive antibody titers (>25<=50) and n=33 for ELISA high positive antibody titers (>50) (Group 1), the average number of S. haematobium eggs found in positives is 1.3 per 10 milliliters urine compared to 0.4 per 10 milliliters urine in the HIV-discordant group (n=99 consecutive HIV discordant couples attending routine follow-up appointments at research clinic) (Group 2). The average number of S. mansoni eggs in positives found in HIV-concordant positive relationships is 11.5 eggs per gram compared to only 4.4 eggs per gram in the HIV discordant group. As expected given the selection criteria for participants in the concordant HIV+ group, an average ELISA titer of 68.9 has been found in Group 1 (Table 1), more than double that of individuals in HIV discordant relationships (32.1). Demographic variables such as average age, years lived in Lusaka and average weights are similar across both groups.

Twenty one percent of individuals in HIV-concordant positive relationships have active infection for schistosomiasis, with 19.1 percent positive for S. haematobium and 7.4 percent positive for S. mansoni. Additionally, 10.3 percent of individuals in HIV-concordant positives are positive for Ascaris lumbricoides. For individuals in HIV-discordant partnerships, 13 percent have active schistosomiasis, with 10 percent positive for S. haematobium, five percent positive for S. mansoni and four percent positive for A. lumbricoides (Table 2). Important differences between groups emerged for having lived in a village or rural area before age 16, stress

incontinence and usage of intravaginal herbs. History of bloody urine within the past three months was found in 13 percent of Group 1 and 10 percent of Group 2.

Of 167 study participants, this study has found 26 to be positive for active schistosomiasis infection (16 percent) and 136 to be negative for active schistosomiasis infection with five individuals missing samples or data for schistosomiasis status. The age range of schistosomiasis positives ranged from 17 to 57 with a mean age of 33 years. Average number of S. haematobium ova excreted in the urine for positives was 5.9 eggs per 10ml urine, while the average number or S. mansoni was found to be 121.2 eggs per gram feces. Mean ELISA titers did not differ for individuals negative for active infection compared with schistosomiasis positive individuals (49.4 versus 42.4, p=NS) (Table 3). Patients with SH and SM eggs were more likely to have low or high positive ELISA titer antibodies than those without schistosome eggs, however these results were non-significant (p=0.3) (Table 4). Similarly, the proportion with positive immunoblot for SH was higher in those with SH or SM eggs compared with egg-negative patients. Interestingly, immunoblot for SM was not associated with the presence of either SM or SH eggs. A significant number of those without active infection also showed the presence of antibodies in ELISA testing (61% for S. haematobium (Sh) and 62% for S. mansoni(Sm)) as well as in immunoblot testing (35% for Sh and 35% for Sm). Individuals positive for schistosomiasis were found to have lived in urban Lusaka for on average four more years than those negative for schistosomiasis. Bivariate analyses found living longer than 15 years in Lusaka, being positive for A. lumbricoides infection and self-reported infertility to be borderline significant at the p<=0.10 level for having active schistosomiasis (Table 5).

Additionally, bivariate analyses were carried out in order to see which variables were significantly associated with HIV serostatus for HIV-positive (n=107) and HIV-negative (n=60).

Positive Western blot result for S. haematobium and belonging to the Nyanja tribe were all very statistically significant associations ($p<0.05$) with having HIV-positive serostatus (Table 6), although this was expected given that all individuals in HIV-concordant positive relationships were selected based on low or high positive titer antibody levels. HIV-positive individuals were found to have greater mean egg load for S. haematobium and S. mansoni than HIV-negative individuals (Table 7). Stratifying measures of schistosome infection by HIV status for only individuals in HIV-discordant couples (n=99) showed 10 percent of HIV positives to have eggs in the urine, the majority (60 percent) to have "negative" ELISA antibody titers yet have more positive immunoblot results for S. haematobium than positive results for S. mansoni (Table 8).

Significant gender differences emerged in analyses of continuous and categorical independent variables by male gender (n=91) and female gender (n=76). Men were found to have greater ELISA antibody titers than women (Table 9) and present with more S. haematobium eggs while women were found to present with more mean S. mansoni eggs and have greater average weight. At the $p<0.05$ level, women had a statistically significant greater likelihood of being less than 30 years of age ($p=0.0004$), being unemployed ($p<.0001$), being born in Lusaka Province ($p=0.0237$) and having little or no understanding of English ($p=<.0001$). Men had a statistically significant greater likelihood of having childhood swimming exposure to schistosomiasis ($p=0.0005$) (Table 10). Measures of schistosome infection stratified by gender are shown in Table 11 with higher prevalence of S. haematobium eggs present in women and men having higher percentage of positive ELISA titers (67% vs. 58%) and higher percentages for immunoblot results across both species.

Comparing ELISA antibody results to immunoblot results yielded some surprising findings with 39 percent of immunoblot results positive for S. haematobium testing negative for

S. haematobium under ELISA testing. Similarly for S. mansoni with 21 percent of positive immunoblot results testing negative on ELISA tests. For all positive immunoblot results (n=87), 30 percent were negative for ELISA antibody titer (Table 12). Univariate odds ratios predicting positive active schistosomiasis are reported in Table 8. Those positive for schistosomiasis infection were found to be 2.38 times the odds of living more than 15 years in Lusaka than individuals negative for schistosomiasis. Additionally, schistosomiasis positives were found to have 6.24 times the odds of being co-infected with A. lumbricoides as those negative for schistosomiasis (although the confidence interval was quite large (1.66, 23.41)). Men and women positive for schistosomiasis were also found to have 4.40 times the odds of self-reported infertility as individuals found to be schistosomiasis negative (Table 13).

In order to simultaneously study the impact of several variables, a multivariate logistic regression model was constructed. Backward elimination was applied (with exclusion criteria of having p-values >0.2) to arrive at a final parsimonious model. Included, as well, in the final model were variables significant in the univariate analysis (Ascaris lumbricoides positive) and variables found to be significantly associated in the literature with having positive active schistosomiasis (History of Childhood Water Exposure, Positive Western blot result for S. haematobium). The final logistic "reduced" model consisted of being less than 30 years of age, having high positive ELISA antibody titer (>=52.7), a history of both bathing and swimming in infected water sources as children, having a positive Western blot result for S. haematobium, living greater than 15 years in Lusaka and testing positive for A. lumbricoides infection (Table 14).

This model had a high Area under the Receiver Operator Curve (AUROC) of 0.8019 (Figure 15) and a statistically significant Likelihood Ratio (0.0060) showing a significant

association between the dichotomous outcome and at least one of the independent variables. However, the overall max r-square value of the model was quite low (0.3285) and the Hosmer and Lemeshow Goodness of Fit Test (HLGOF) (used to test how well the predicted frequency of an outcome (active schistosomiasis) matches the observed frequency) was 0.6340 with a chi-square value of 5.21 (Table 15). Low chi-square values and high values of for HLGOF generally indicate an acceptable fit for the model and observed values. Tests for interaction yielded no statistically significant interaction terms at the $p<0.05$ level, tests of multicollinearity between the independent predictor variables yielded VIF values of less than three for all variables and examinations of the deviance residuals (Figure 6) yielded no absolute values considered outliers.

Surprising results were found in bivariate analyses for schistosomiasis status and HIV serostatus as well as some results which were expected to be significant but were found to be non-significant or underreported. The extremely low number of STIs, genital abnormalities, vaginal and penile discharge as well as the low levels of reported erectile dysfunction, painful ejaculation and bloody urine were surprising. Statistically significant associations (at the $p<0.10$ level) between testing positive for schistosomiasis and positive A. lumbricoides infection ($p=0.0105$) and infertility ($p=0.0797$) were unexpected and may hint at significant polyparasitism in this population. Belonging to the Nyanja tribe, having high ELISA schistosomiasis antibody titer ($p<.0001$) and a positive Western blot result for S. haematobium (Table 6) were all statistically associated with being HIV positive in this population. Bloody urine was found to not be significantly associated with having active schistosomiasis ($p=0.9170$) as well as living in a rural area or village before age 16 ($p=0.4241$) despite being shown in other studies to be significant predictors of schistosomiasis infection.

In this cross-sectional study, 21 percent of HIV-positive individuals in concordant positive relationships were found to have active schistosomiasis, with 19 percent positive for S. haematobium. For individuals in HIV-discordant relationships, 13 percent were positive for active schistosomiasis, with 10 percent positive with S. haematobium in the urogenital tract. Years lived in Lusaka, being co-infected with A. lumbricoides and self-reported infertility were all statistically significant ($P<0.10$) predictors of positive schistosomiasis infection under bivariate analyses. The logistic regression model developed contained the variables of age<30 years, high positive ELISA antibody titer (>=52.7), childhood water exposure, positive Western blot for S. haematobium, years lived in Lusaka and A. lumbricoides co-infection. The model had an overall ability to discriminate between schistosomiasis positives and negatives (AUROC) of 0.8019 and a statistically significant Goodness of Fit p-value of 0.6340, however the r-squared value was low (0.3285) meaning that only about 33 percent of the differences between positive and negative schistosomiasis status could be explained by the independent variables in the regression model.

CHAPTER 4: DISCUSSION

This cross-sectional study shows active schistosomiasis to be a common infection in both HIV positive individuals in concordant positive relationships (21 percent prevalence) and in individuals in HIV discordant relationships (13 percent). It shows that individuals who have lived in the urban capital more than 15 years, Lusaka, were 2.38 times as likely to have active schistosomiasis as those who have lived less than 15 years. Individuals infected with the A. lumbricoides parasite are six times as likely to be co-infected with schistosomiasis as those negative for A. lumbricoides. Furthermore, those infected with active schistosomiasis are four times as likely to report infertility as individuals negative for schistosomiasis. The logistic regression model developed for this study validates the importance of young age, high antibody titer, childhood water exposure, positive Western blot result, peri-urban residence and polyparasitism as predictive factors for testing positive for schistosomiasis.

Many of my findings are supported by the literature around schistosomiasis, HIV and public health and may help to explain some of the unexpected and unusually low values found for some predictive variables. As Kjetland has shown in Zimbabwe, urogenital schistosomiasis can be found in 58 percent of women with ova in the urine but also in 41 percent of women without ova in the urine (Kjetland 2005). Thus, both due the fact that only one urine sample was collected per individual and that urogenital schistosomiasis can be diagnosed in women without ova excretion in the urine, my figures of urogenital prevalence are undoubtedly an underestimation. Similar prevalence of S. haematobium (12.3%) was found in Nigeria by Ukwandu and shown to be age-dependent and influenced by exposure to cercariae-infected water (Ukwandu 2004) (similarly to my logistic regression model).

For HIV positive individuals (n=107), this study found a greater mean S. haematobium egg excretion (6.2 per 10ml urine) than other studies in Tanzania (Poggensee 1998 with an average of 2.2/10ml urine, though with a much larger sample size, n=543) and Malawi (Kjetland 1996, 5/10ml urine, n=51). Findings that being less than 30 years of age being statistically associated with having active schistosomiasis are supported by Kjetland's findings of higher egg loads in younger women (Kjetland 1996) and that urinary S. haematobium infection peaks in the early twenties and falls to lower levels after age 30 (Kjetland 2012). These findings that adult HIV serostatus does not impair the ability to excrete eggs in low-intensity infections and that co-infection with HIV does not negatively affect diagnosis and surveillance for schistosomiasis support earlier work by Kallestrup (Kallestrup 2005).

Surprising findings that hematuria was not a good predictor of active schistosomiasis (p=0.9170) are supported by Doenhoff (Doenhoff 2004) and Poggensee (Poggensee 2000a) who found, in a study of 303 women in Tanzania, that only 54 percent of hematuria cases could be explained as S. haematobium. They note that hematuria increases with age in women regardless of urogenital schistosomiasis status (Poggensee 2000a) and that trace blood in the urine could be due to menstruation or other genital infection. Finally, the utility of questionnaires used in this study to assess symptoms and predictors of schistosomiasis in adults validated results for ethnic differences and childhood water exposure (Kjetland 2005, Danso-Appiah 2010, Pinot de Moira 2011) and age and infertility (Ukwandu 2004).

High percentages of both active schistosomiasis cases and positive immunoblot test findings resulting in negatives under ELISA (<25) speak to the numerous limitations outlined in the methodology of this study. The designation of a positive cut-off for ELISA antibody titer was made using standard deviations of negative controls but this designation is ultimately an

artificial one made by the researcher to impose an arbitrary dichotomous nature on a continuous variable (antibody titer). Criticisms of ELISA antibody testing as lacking sensitivity and robustness also mean that antibody positives could have been missed. Lastly, not all samples had matching ELISA and Immunoblot results and there were missing data which undoubtedly affected the results and high negative results for those positive for active schistosomiasis.

CONCLUSIONS/IMPLICATIONS

A quote from a colonial era doctor in Northern Rhodesia (now Zambia) in 1937 (as paraphrased in Michelson 1989) seems as telling today as it was 75 years ago as to how much is left unknown about schistosomiasis in endemic countries. He stated "each year it becomes clearer the infection (schistosomiasis) is commoner than has been realized, that the infection is frequently present without causing recognized symptoms and that medical textbooks and instruction give a very incomplete picture of the disease which leads many cases to be overlooked" (Michelson 1989). Due to widely varying assessments of schistosomiasis prevalence in Zambia (a country endemic for both HIV-1 and schistosomiasis), this study was undertaken to capture a snapshot of the prevalence of both S. haematobium and S. mansoni in HIV positive individuals and HIV-negative individuals in HIV discordant relationships.

Due to the temporal, financial and methodological limitations inherent in carrying out this cross-sectional study, it may be assumed that the observed prevalence of schistosomiasis was an underestimation of the true prevalence in the study population. However, the fact that any adults were found to have active excretion of urinary schistosomiasis ova and intestinal ova, given limited diagnostic means, was an important finding. In this study population, 16 percent of all participants were schistosomiasis positive for active infection with 19 percent of HIV-positive

individuals co-infected with schistosomiasis. This research adds to a growing body of literature that active schistosomiasis infection is an important parasitic disease in Zambia and should be tested for and treated in populations of HIV-infected individuals and HIV-negative individuals in HIV-discordant intimate partnerships.

Without the ability to follow these HIV negative individuals infected with schistosomiasis longitudinally for seroconversion, it is impossible to make cause and effect determinants from these findings on the possible line between S. haematobium and HIV-1 infection/transmission. Though this research adds to the circumstantial evidence suggestive of a biological link, much more laboratory and epidemiological work needs to be done (Recommendations).Additionally, this study supports Pinot de Moira's (Pinot de Moira 2011) finding that top-down worm control programs (such as those against schistosomiasis) cannot be sustainable without understanding the local socio-ecological conditions of a country, its demographics, its exposure pathways, its environmental reservoirs and local prevalence.

More broadly, these findings demand that public health reconsider our approach to HIV prevention and consider the impact that cost-effective treatments and testing for parasitic cofactors may have on HIV-1 transmission. It is clear that "endemic parasites and infectious diseases (such as schistosomiasis) are not just background noise" (Sawers 2008) in areas of HIV endemicity. These cofactors increase the likelihood of HIV infections and may alter the dynamics of epidemic spread and thus antihelminthic drug prophylaxis and treatment (at 40 cents per treatment) bear consideration, especially in the current era of fiscal restraint and achieving more in global health with less. Additionally, these findings have important implications for current STD policy in sub-Saharan Africa, as many men and women may currently be misdiagnosed and treated for STDs when in fact they have symptoms of genital schistosomiasis.

This unnecessary and potentially harmful treatment (WHO 2009) can be prevented through implementing diagnostic procedures (such as those described in this study) and making training and equipment available for health care workers, doctors, nurses and OBGYNs in endemic countries such as Zambia.

This research supports Rwanda Zambia HIV Research Group's (and their in-country site, Zambia Emory HIV Research Project) continued mission to better understand the dynamics of HIV-1 transmission in Zambia, to train Zambia's health care workers, nurses and laboratory technicians and to deliver the best possible care and referrals to care for HIV-concordant positive and HIV-discordant couples in Zambia. Following this research, a ZEHRP-run and International AIDS Vaccine Initiative (IAVI) funded vaccine trial in Lusaka tested participants receiving the vaccine and found 4 out of 23 (17.4%) to be positive for S. haematobium. This is undoubtedly an underestimate of the prevalence of HIV vaccine recipients as urine filters for syringe urine filtration ran out halfway through the vaccine trial. More filters were subsequently shipped to Zambia to continue routine testing.

Additionally, the finding of significant prevalence of schistosomiasis infection in adults has been incorporated into ZEHRP's roll-out of a "good health package". This suite of medical services (also including mosquito nets, chlorine water purification, soap and treatment of intestinal worms) has been offered to couples in longitudinal studies in the Copperbelt Province of Zambia as non-monetary incentives to increase rates of follow-up. Only very recently initiated, four couples have since been tested for urogenital schistosomiasis and in the first week of being offered, cost-effective treatment for intestinal worms (including S. mansoni) and treatment for syphilis increased follow-up rates for couples from 15 to 55 percent (Susan Allen, personal communication). These findings of active schistosomiasis will also be used next year to

examine male genital schistosomiasis in this population through comparisons of ELISA and active schistosomiasis positive men with banked semen samples in order to test the semen samples for HIV viral load.

RECOMMENDATIONS

This study shows clearly that there is significant prevalence of schistosomiasis in urban adults living in a country co-endemic for HIV-1, S. haematobium and S. mansoni. This finding necessitates a shift in thinking of schistosomiasis from being simply a disease afflicting children in rural areas. There must be increased recognition of schistosomiasis, both urogenital and intestinal, afflicting urban and peri-urban populations and more studies carried out in these populations who have been traditionally perceived to lack risk factors of waterborne disease. However, as has been shown in peri-urban Lusaka, individuals in areas without effective water delivery systems practice a "hybridization of urban and rural models of water supply infrastructure" (Agnew-Blais 2010). This may mean exposure to Schistosoma parasites in streams, effluent from dams and ponds in peri-urban areas for women and children, especially. More research is needed to examine the role of urbanization and migrant movements in schistosomiasis transmission, especially in endemic African countries lacking established water infrastructure.

More longitudinal studies following couples and individuals with schistosomiasis for seroconversion are necessary in order to "confirm the suggested causality of schistosomiasis in incident HIV infection" (Mbabazi 2011). However, at present, more could be done to improve routine testing of blood, urine and feces from HIV-infected individuals and the HIV-negative partner in HIV-discordant relationships in order to better understand how schistosomiasis affects HIV status or acquisition. Including testing for parasitic infections as part of standard HIV care

should not have to wait until the "ideal study" is carried out, we have more than enough reasons to treat worms in immune-compromised individuals (Sawers 2008) and the safety and effectiveness of praziquantel have shown that deworming can decrease HIV viral load and decrease transmission probability (Borkow 2007).

In order to improve the ability of laboratory technicians, doctors, nurses and OBGYNs in resource limited countries to detect schistosomiasis (particularly in the urogenital tract), more training and tools need to be made available and swiftly put into practice. Colposcopy has been shown to be extremely effective at detecting the characteristic lesions, abnormal blood vessels and sandy patches present in female genital schistosomiasis. Provided machines could be made available (especially with computerized digital imaging), OBGYNs would be able to have a clear, illuminated view of the vagina, cervix and epithelium tissue, replacing crude techniques of using magnifying glasses, flashlights and stains. Not only would improvements in gynecological care benefit diagnosis and treatment of schistosomiasis but could also help to diagnose sexually transmitted infections earlier as well be vital in cervical cancer prevention. The introduction of ultrasound technology (WHO 2009) and schistosomiasis PCR (shown to be 100 percent specific (Kjetland 2009) can prove extremely beneficial at reducing morbidity and mortality, provided local staff is trained in their usage and not simply used by Western researchers and then left. Furthermore, the reagent assay, validated in Tanzania (Van Dam 2004) may be especially useful in Zambia with its 90 percent specificity, its ability to be used without technical training or equipment and to last up to three months in ambient temperatures without degrading.

Parasitological testing and treatment has a place in HIV prevention and care programming and as such, mass drug administration of praziquantel should be integrated into existing PEPFAR and GFATM structures in endemic countries. Considering PEPFAR and

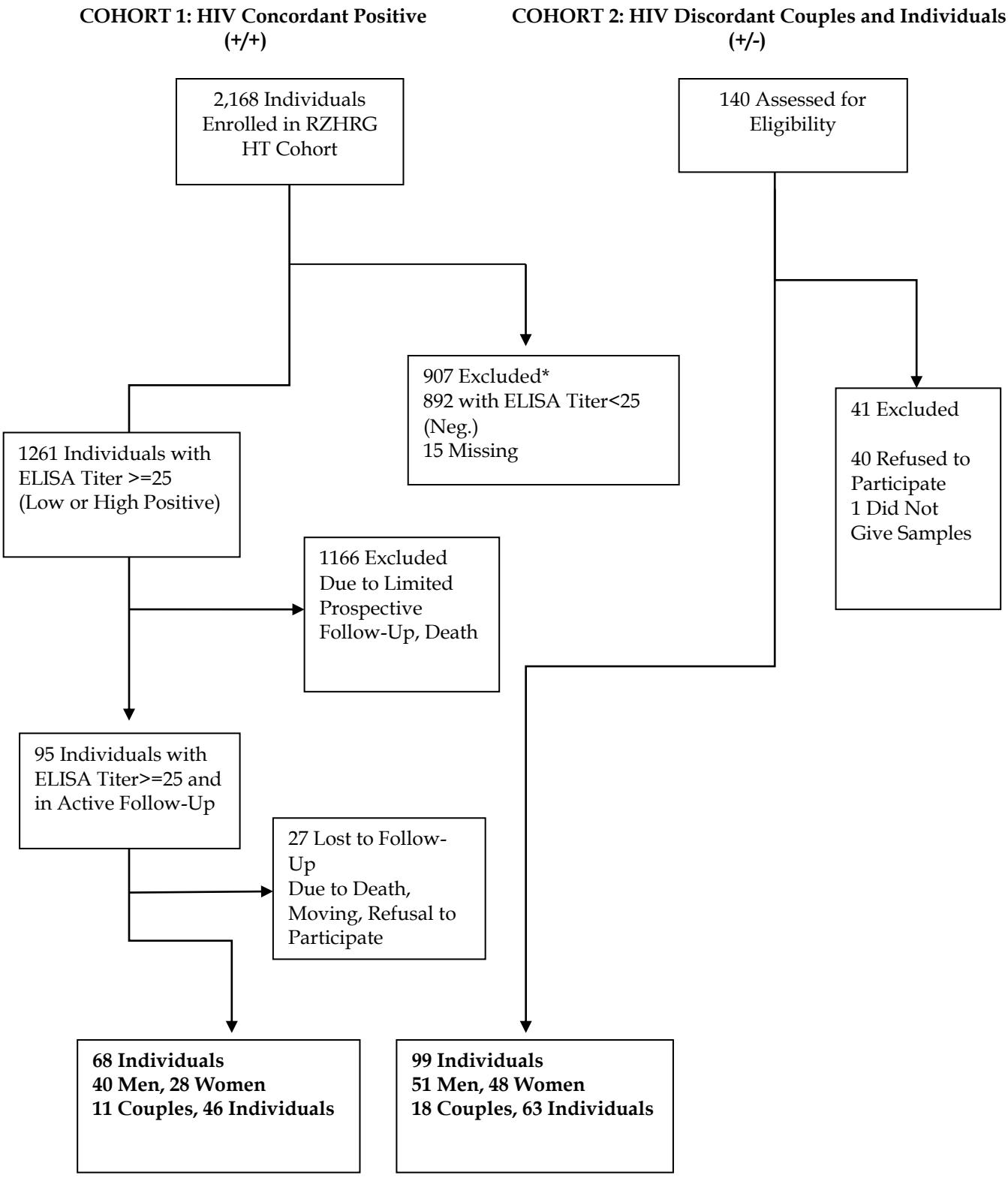
GFATM funds already support treatment for other co-infections such as tuberculosis and opportunistic infections, why can't praziquantel chemotherapy also be considered? This cheap, effective drug also must be targeted as prophylaxis at young women (especially under 30 years of age (Results)) and girls, the groups most at risk for HIV-1 acquisition. Girls who receive frequent and periodic praziquantel treatment are "less likely to acquire HIV, pregnant women are less likely to pass on HIV to their fetus and preventive chemotherapy may reduce HIV transmission" among girls and young women by reducing viral loads (Hotez 2007). With this approach, praziquantel dosage, effectiveness and limitations must be adequately explained to the patient with the distinction being made that the drug will cure mature worms and eliminate eggs but not remove lesions and genital abnormalities.

However, operating simply under a test and treat mindset ignores the crucial role that vector control can have in eliminating Schistosoma parasites from the environment in which individuals live and interact with water bodies. Although many adults in this study were most likely infected as children, exposure to schistosomiasis can occur on a daily basis in peri-urban and rural areas of Lusaka, with its many steams, ponds, dams and sewage runoff features. Giving someone praziquantel and sending them out to become re-infected in the environment is nonsensical (and can lead to more severe clinical outcomes) when one considers the benefits of joining a vector control program with praziquantel prophylaxis and/or treatment. As shown by Remais (Remais in press), sanitation improvements, sewerage, piped water and application of anti-molluscicide to kill infected snails can also supplement and strengthen progress made by the MDA of praziquantel.

Schistosomiasis is no longer the rural pediatric condition it once was viewed to be, the parasitic disease infects both the genital and intestinal tract and can create lesions, bleeding and

immunological changes which make HIV acquisition/transmission more likely. Schistosomiasis was prevalent in 21 percent of HIV-positive individuals in HIV-concordant positive relationships and prevalent in 13 percent of individuals in HIV-discordant relationships. S. haematobium was more common in this population and HIV positive individuals were found to have higher average S. haematobium egg counts versus HIV-negative individuals under syringe urine filtration. Young age, duration of time in Lusaka, antibody titer level and co-infection with A. lumbricoides were all factors associated with having active schistosomiasis. Bloody urine and genital conditions (erectile dysfunction, stress incontinence, vaginal discharge, spot bleeding) were ultimately found to be imperfect, subject to bias and not predictive of schistosomiasis infection. Future research needs to examine the role of male genital schistosomiasis through testing of semen samples as well as continually improve the materials and support to OBGYNs and nurses in Zambia in order to better examine, diagnose and treat genital schistosomiasis. Mass drug administration of praziquantel both as prophylaxis and treatment should be scaled up and integrated into HIV, malaria and other infections disease programs. However, drug administration must be balanced with vector control and elimination of snails in infected water sources as well as investments in water supply and sewerage infrastructure in order to truly eliminate schistosomiasis as a cause of morbidity and mortality for the next generation of Zambians.

Figure 1: Study Flow



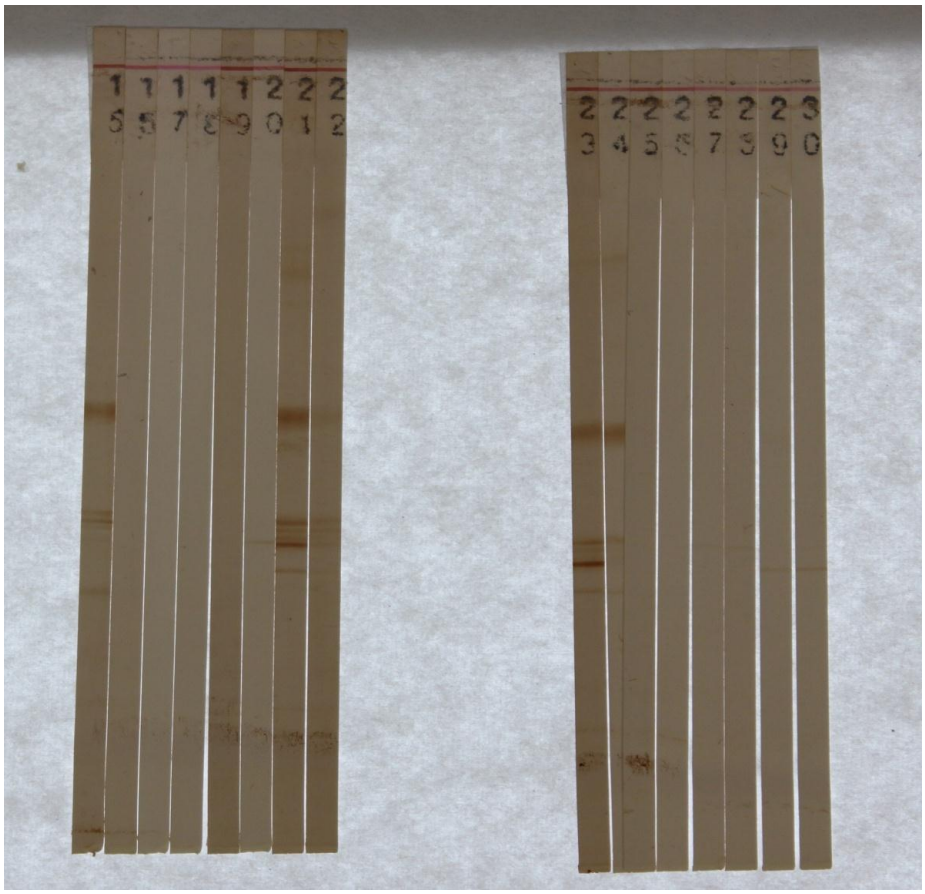


Figure 2: Schistosoma mansoni Western Blot Strips (Positives clearly shown in dark bands of 21 and 23)

Figure 3: Schistosoma haematobium Western Blot Strips (Positives shown in dark bands of strips 17, 43 and 46)

Figure 4: Study Questionnaire

Date_____
Initials_____

HTID_____Sex_____

Questionnaire for Patients:

1. If Female, do you have: Currently:
   a. Frequent Urination (more than once Every two hours and at night) Y N [ ]
   b. Stress Incontinence- (Uncontrollable Urination when laughing, sneezing Or coughing) Y N [ ]
   c. Spot Bleeding (on underwear Or sheets) Y N [ ]
   d. Use of Intravaginal herbs Y N [ ]
   e. Infertility (inability to produce children in past Three years) Y N [ ]
   f. History of Bloody Urine Y N [ ]
   g. Have you had any Sexually Transmitted Infection Within the past 3 months? Y N [ ]

2. If Male, do you have:
   a. Erectile Dysfunction (Unable to achieve Erection) Y N [ ]
   b. Painful Ejaculation Y N [ ]
   c. Infertility (inability to produce children in past three years Y N [ ]
   d. Bloody Urine Y N [ ]
   e. Have you had any Sexually Transmitted Infection Within the past 3 months? Y N [ ]

3. Place of Birth

4. Childhood Water Exposure (choose all that apply)
   a. Swimming [ ]
   b. Bathing/Washing [ ]
   c. River [ ]
   d. Pond [ ]
   e. Lake [ ]
   f. Did people used to urinate and defecate in this water?

5. Current Area in Which You Live

6. Where Do You Get Your Water for Washing, Cooking and Drinking?

Figure 5: AUROC Value of Logistic Regression Model

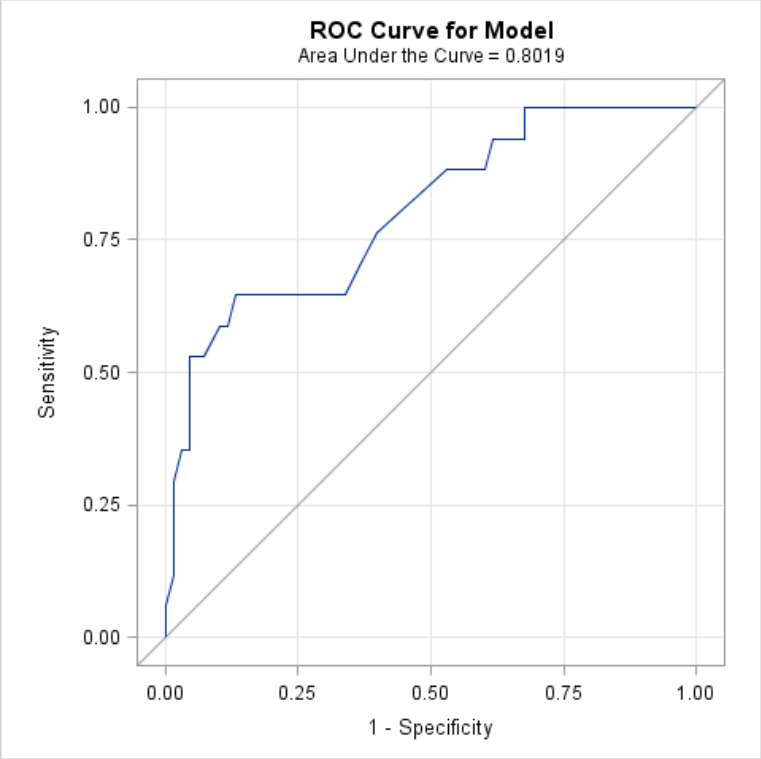
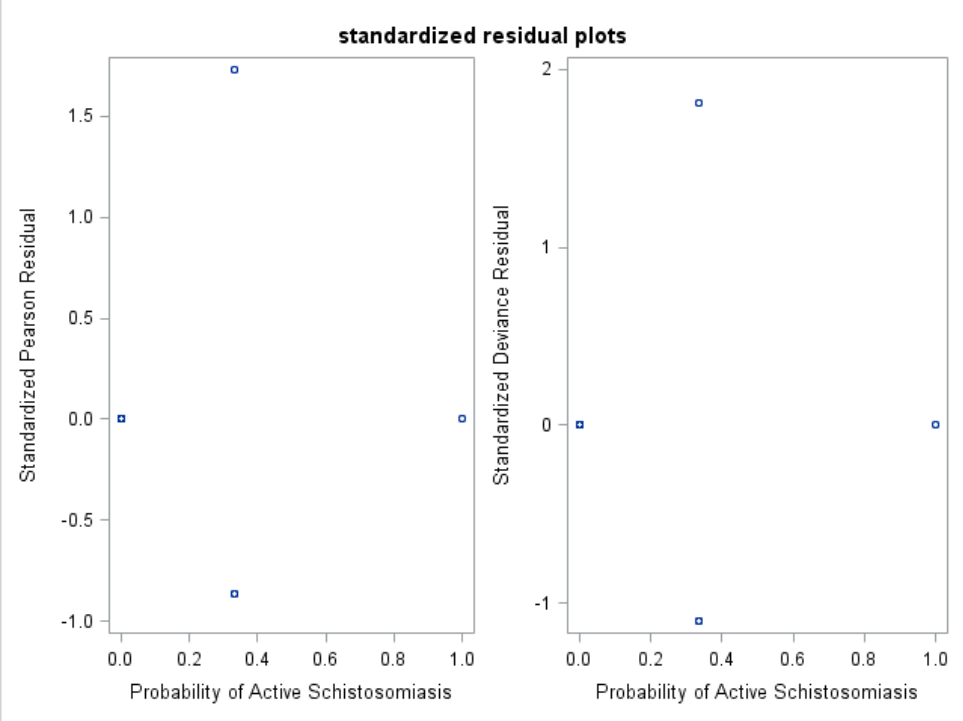


Figure 6: Plot of the Standardized Residuals



Tables and Appendices

Table 1: Descriptive Statistics: Continuous Variables

Follow-Up Individuals in HIV Concordant Positive Partnerships (n=68)

Variable	Distribution Mean (SD)	# Missing	Min, Max
Age	32.1 (7.5)	0	18, 57
S. haematobium Eggs per 10ml Urine (Positives)	1.3 (3.4)	0	0, 19
S. mansoni Eggs per gram Feces (Positives)	11.5 (44.2)	0	0, 240
ELISA Antibodies	68.9 (56.7)	0	25.3, 354.3
Avg. Weight Over Past 3 Months (kg)	61.7 (11.4)	14	41, 99.5
Years Lived in Lusaka	20.5 (13.1)	19	1, 49

Incoming Individuals in HIV Discordant Partnerships (n=99)

Variable	Distribution Mean (SD)	# Missing	Min, Max
Age	32.8 (7.4)	2	17, 50
S. haematobium Eggs per 10ml Urine (Positives)	0.4 (1.4)	0	0, 10
S. mansoni Eggs per gram Feces (Positives)	4.4 (19.5)	0	0, 120
ELISA Antibodies	32.1 (42.9)	12	0.87, 215
Avg. Weight Over Past 3 Months (kg)	57.7 (4.5)	88*	53.7, 67.6
Years Lived in Lusaka	19.7 (13.4)	7	1, 48

***Weight Data stopped being collected early in the study**

Table 2: Descriptive Statistics by Cohort: Categorical Variables

Variable (%)	Follow-Up HIV Concordant Positive (n=68) (%)	Incoming HIV Discordant Cohort (n=99) (%)
Sex (female)	28 (41)	48 (49)
Age<=30 years	32 (47)	40 (41)
>15 years in Lusaka (n=141)	30 (61)	51 (55)
Couples in Cohort	11 couples (32)	18 couples (36)
Positive for Active Schistosomiasis Infection	14 (21)	12 (13)
Positive for Ascaris lumbricoides Infection	7 (10)	4 (4)
HIV Positive Serostatus	68 (100)	39 (39)
High ELISA Titer (>=52.7)	32 (47)	15 (17)
Positive Western Immunoblot Result for S. haematobium	11 (39)	33 (36)
Positive Western Immunoblot Result for S. mansoni	10 (36)	20 (22)
Born in Lusaka Province	25 (37)	35 (35)
Childhood Swimming Exposure	51 (86)*	52 (61)*
Exposure in River Polluted by Human Waste	40 (83)	61 (91)
Childhood Water Source Used by Community for Urination and Defecation	52 (98)	77(93)
Currently Lives in Lusaka Central	26 (41)	30 (31)
Unemployed	12 (25)	41 (45)
Lived in Village or Rural Area before Age 16	17 (35)	49 (53)
Little or No English Literacy	25 (51)	40 (43)
Erectile Dysfunction (Men)	6 (16)	3 (6)
Painful Ejaculation (Men)	2 (5)	3 (6)
Nyanja Tribe	23 (34)	41 (41)
Frequent Urination	6 (9)	6(6)
Stress Incontinence (Women)	6 (22)	5(10)
Spot Bleeding (Women)	1 (4)	0(0)
Usage of Intravaginal Herbs (Women)	9 (33)	8 (17)
Infertility	4 (6)	3(3)
Bloody Urine	8 (13)	10(10)
Sexually Transmitted Infection in Past 3 Months	5 (8)	1 (1)

*>20% missing

Table 3: Descriptive Statistics: Continuous Variables

Positive for Active Schistosomiasis Infection (n=26)

Risk Factor	Distribution Mean (SD)	# Missing	Min, Max
Age	33.1 (9.4)	0	17, 57
S. haematobium Eggs per 10ml Urine (Positives)	5.9 (4.24)	5	2, 19
S. mansoni Eggs per gram Feces (Positives)	121.2 (59.8)	16	72, 240
ELISA Antibodies	42.4 (42.3)	1	3.77, 214.9
Years Lived in Lusaka	23.5(12.3)	3	1, 47

Negative for Active Schistosomiasis Infection (n=136)

Risk Factor	Distribution Mean (SD)	# Missing	Min, Max
Age	32.4(7.0)	2	18, 50
ELISA Antibodies	49.4 (55.1)	10	0.87, 354.3
Years Lived in Lusaka	19.5 (13.5)	25	1, 49

Table 4: ELISA and Immunoblot Results in Patients With and Without Detectable S. haematobium and S. mansoni Eggs

	Active S. haematobium		MISSING	Active S. mansoni		MISSING	BOTH		MISSING	NEITHER		MISSING
	N	**%**	N (%)	**N**	**%**	N (%)	**N**	**%**	N (%)	**N**	**%**	**N (%)**
ELISA antibody titer												
<25 (Negative)	5	25%	1 (5)	3	30%	0	7	28%	1 (4)	49	39%	10 (8)
26-50 (Low Positive)	11	55%		2	20%		11	44%		37	29%	
>50 (High Positive)	4	20%		5	50%		7	28%		40	32%	
Positive Immunoblot for S. haematobium												
yes	7	47%	6 (29)	5	56%	1 (10)	9	45%	6 (30)	34	35%	39 (40)
no	8	53%		4	44%		11	55%		63	65%	
Positive Immunoblot for S. mansoni												
yes	3	20%	6 (29)	1	11%	1 (10)	3	15%	6 (30)	27	28%	39 (40)
no	12	80%		8	89%		17	85%		70	72%	

Table 5: Bivariate Table for Active Schistosomiasis: Categorical Variables				
Variable	Positive for Active Schistosomiasis Infection (n=26) (%)	Negative for Active Schistosomiasis Infection (n=141) (%)	# Missing	Chi-Square P-Value
Sex (female)	12 (46)	63 (46)	5	0.9873
Incoming Discordant	12 (46)	84 (61)	5	0.1377
Age<=30 years	13 (50)	57 (43)	7	0.4827
>15 Years in Lusaka	17 (74)	62 (54)	30	**0.0838**
HIV Positive Serostatus	19 (73)	83 (61)	5	0.2438
Positive Ascaris lumbricoides Infection	5 (19)	5 (4)	5	**0.0105***
High ELISA Titre (>=52.7)	7 (28)	38 (30)	16	0.2588
Positive Western Immunoblot Result for S. haematobium	9 (45)	34 (34)	50	0.4008
Positive Western Immunoblot Result for S. mansoni	3 (15)	27 (28)	50	0.2714*
Born in Lusaka Province	13 (50)	45 (33)	5	0.3117*
Childhood Swimming and Bathing Exposure	19 (83)	81 (69)	27	0.3117*
Exposure in River Polluted by Human Waste	12 (46)	85 (60)	56	1.0000*
Childhood Water Source Used by Community for Urination and Defecation	21 (81)	104 (74)	35	0.9041
Currently Lives in Lusaka Central	10 (40)	43 (33)	10	0.4717
Unemployed	11 (48)	42 (37)	30	0.3238
Lived in Village or Rural Area before Age 16	9 (39)	55 (48)	30	0.4241
Little or No English Literacy	14 (61)	50 (44)	30	0.1358
Erectile Dysfunction (Men (n=13)	3 (23)	6 (8)	83	0.1402*
Painful Ejaculation (Men (n=13)	1 (8)	4 (6)	83	0.5783*
Nyanja Tribe	13 (50)	47(35)	5	0.3254
Frequent Urination	1 (4)	11 (8)	93	0.8297*
Stress Incontinence (Women (n=12)	2 (17)	9 (15)	93	1.0000*
Spot Bleeding (Women)	0(0)	1 (2)	93	1.0000*
Usage of Intravaginal Herbs (Women(n=12)	2 (17)	15 (24)	93	0.7212*
Infertility	3 (12)	4 (3)	9	**0.0797***
Bloody Urine	3 (12)	15 (11)	9	0.9170
Sexually Transmitted Infection in Past 3 Months	0(0)	6(5)	9	0.5906*

*Fisher's Exact Test: Two-Sided P-Value
Bold=Significant at p<0.10

Table 6: Bivariate Analyses by HIV Serostatus				
Variable	HIV Positive (n=107) (%)	HIV Negative (n=60) (%)	# Missing	Chi-Square P-Values
Sex (female)	45 (42)	31 (52)	0	0.2315
Age<=30 years	46 (43)	26 (44)	2	0.9336
>15 Years in Lusaka	47 (56)	34 (60)	26	0.6631
Active Schistosomiasis Infection	19 (19)	7 (12)	5	0.2438
Positive Ascaris lumbricoides Infection	8 (7)	3 (5)	0	0.7478*
High ELISA Titer (>=52.7)	40(39)	7 (13)	12	**<.0001**
Positive Western Immunoblot Result for S. haematobium	29 (48)	15 (25)	47	**0.0120**
Positive Western Immunoblot Result for S. mansoni	17 (28)	13 (22)	47	0.4605
Born in Lusaka Province	37 (35)	23 (38)	0	0.7066*
Childhood Swimming and Bathing Exposure	70 (75)	33 (63)	22	0.1328
Exposure in River Polluted by Human Waste	62 (86)	39 (91)	52	0.4699*
Childhood Water Source Used by Community for Urination and Defecation	82 (95)	47 (94)	31	0.7912*
Currently Lives in Lusaka Central	40 (39)	16 (27)	5	0.1048
Unemployed	27 (32)	26 (46)	26	0.1051
Lived in Village or Rural Area before Age 16	38 (45)	28(49)	26	0.6501
Little or No English Literacy	42 (50)	23 (40)	26	0.2593
Erectile Dysfunction (Men)	8 (14)	1 (3)	79	0.2613*
Painful Ejaculation (Men)	1 (8)	4 (6)	83	1.0000*
Nyanja Tribe	38 (36)	26(43)	0	**0.0147***
Frequent Urination	8 (18)	4 (13)	1	0.7508
Stress Incontinence (Women)	8 (18)	3 (10)	92	0.3457
Spot Bleeding (Women)	1(2)	0 (0)	92	1.0000
Usage of Intravaginal Herbs (Women)	12 (27)	5 (16)	92	0.4014*
Infertility	5 (5)	2 (3)	4	1.0000*
Bloody Urine	13 (13)	5 (8)	4	0.4500*
Sexually Transmitted Infection in Past 3 Months	5(5)	1(2)	4	0.4153*

*Fisher's Exact Test: Two-Sided P-Value
Bold=Very Significant at p<0.05

Table 7: Descriptive Statistics by HIV Serostatus

HIV Positive Individuals (n=107)

Variable	Distribution Mean (SD)	# Missing	Min, Max
Age	32.4 (7.5)	1	17, 57
S. haematobium Eggs per 10ml Urine (n=17 Positives)	6.2 (4.6)	0	2, 19
S. mansoni Eggs per gram Feces (n=7 Positives)	132 (68.7)	0	72, 240
ELISA Antibodies	57.4 (54.1)	4	2.56, 354.3

HIV Negative Individuals (n=60)

Variable	Distribution Mean (SD)	# Missing	Min, Max
Age	32.7 (7.1)	1	19, 48
S. haematobium Eggs per 10ml Urine (n=4 Positives)	4.3 (1.5)	0	2, 5
S. mansoni Eggs per gram Feces (n=3 Positives)	96.0 (24)	0	72, 120
ELISA Antibodies	30.1 (42.9)	8	0.87, 215

Table 8: Measures of Schistosome Infection Stratified by HIV Status (Individuals in Discordant Couples) (n=99)

	HIV+ (n=39)		MISSING	**HIV- (n=60)**		MISSING
	N	%	N (%)	**N**	%	N (%)
Schistosome eggs in urine						
Yes	4	10%	0	4	7%	0
No	35	90%		56	93%	
Schistosome eggs in stool						
Yes	2	5%	0	3	5%	0
No	37	95%		57	95%	
ELISA antibody titer						
<25	21	60%	12 (14)	36	69%	12 (14)
26-50	5	14%		9	17%	
>50	9	26%		7	13%	
Positive immunoblot for Sh						
Yes	18	55%	7 (8)	15	25%	7 (8)
No	15	45%		44	75%	
Positive immunoblot for Sm						
Yes	7	21%	7 (8)	13	22%	7 (8)
No	26	79%		46	78%	

Table 9: Descriptive Statistics by Gender

Men (n=91)

Variable	Distribution Mean (SD)	Min, Max
Age	35.0 (7.4)	21, 57
S. haematobium Eggs per 10ml Urine (Positives)(n=10)	5.9 (5.7)	2, 19
S. mansoni Eggs per gram Feces (Positives)(n=7)	104.6 (43.8)	72, 194
ELISA Antibodies	55.9 (61.5)	0.87, 354.3
Avg. Weight Over Past 3 Months (kg)	59.0 (7.4)	46.8, 79.0

Women (n=76)

Variable	Distribution Mean (SD)	Min, Max
Age	32.8 (7.4)	17, 50
S. haematobium Eggs per 10ml Urine (Positives)(n=11)	5.2 (2.6)	3, 10
S. mansoni Eggs per gram Feces (Positives)(n=3)	160 (84.3)	72, 240
ELISA Antibodies	38.9 (37.4)	2.01, 191.2
Avg. Weight Over Past 3 Months (kg)	63.6 (13.3)	41, 99.5

Table 10: Bivariate Analyses for Gender

Variable	Male (n=91) (%)	Female(n=76) (%)	# Missing	Chi-Square p-value
Positive for Active Schistosomiasis	14 (16)	12 (16)	5	0.9873
<=30 years of age	28 (31)	44 (59)	2	**0.0004**
>15 years in Lusaka	45 (59)	36 (55)	26	0.6469
HIV Positive Serostatus	62 (68)	45 (59)	0	0.2315
Ascaris lumbricoides Positive	7 (8)	4 (5)	0	0.7558*
High Positive ELISA Titer (>52.7)	31 (36)	16 (23)	12	0.1356
Positive Western blot Result for S. haematobium	27 (47)	17 (27)	47	**0.0298**
Positive Western blot Result for S. mansoni	16 (28)	14 (23)	47	0.5269
Born in Lusaka Province	28 (31)	32 (42)	0	**0.0237**
Childhood Swimming Exposure	67 (83)	36 (56)	22	**0.0005**
Lives in Lusaka Central	32 (37)	24 (32)	5	0.6002
Unemployed	7 (9)	46 (71)	26	**<.0001**
Lived in Rural Area or Village before Age 16	39 (51)	27 (42)	26	0.2461
Little or No Understanding of English	20 (26)	45 (69)	26	**<.0001**
Nyanja Tribe	38 (42)	26 (34)	0	0.4622
Childhood Water Source Used by Community for Urination and Defecation	73 (92)	56 (98)	31	0.4004*
Self-Reported Infertility	4 (5)	3 (4)	4	1.0000*
Bloody Urine	13 (15)	5 (7)	4	0.0998
STI in Past Three Months	5 (6)	1 (1)	4	0.2191*

*Fisher's Exact Test: Two-Sided P-Value

Bold=Very Significant at p<0.05

Table 11: Measures of Schistosome Infection Stratified by Gender

	Male		MISSING	Female			All		MISSING
	N	**%**	N (%)	**N**	**%**		**N**	**%**	N (%)
Schistosome eggs in urine									
Yes	10	11%	0	11	14%		21	13%	0
No	81	89%		65	86%		146	87%	
Schistosome eggs in stool									
Yes	7	8%	0	3	4%		10	6%	0
No	84	92%		73	96%		157	94%	
ELISA antibody titer									
<25	28	33%	6 (7)	29	41%		57	37%	6 (8)
26-50	25	29%		24	34%		49	32%	
>50	32	38%		17	24%		49	32%	
Positive immunoblot for SH									
Yes	27	47%	33 (36)	17	27%		44	37%	14 (18)
No	31	53%		45	73%		76	63%	
Positive immunoblot for SM									
Yes	16	28%	33 (36)	14	23%		30	25%	14 (18)
No	42	72%		48	77%		90	75%	

Table 12: ELISA Results Compared to Immunoblot Results

	POSITIVE IMMUNOBLOT RESULTS							
ELISA	S. haematobium		S. mansoni		Both		Neither	
negative	17	39%	6	21%	3	20%	36	65%
low pos	9	20%	11	39%	5	33%	14	25%
high pos	18	41%	11	39%	7	47%	5	9%
			Missing: 2 (7%)				**Missing: 6 (10%)**	
	Positive Immunoblot Results							
ELISA	Yes	%	No	%				
negative	26	30%	36	65%				
low pos	25	29%	14	25%				
high pos	36	41%	5	9%				

Table 13: Univariate Odds Ratios as Predictors of Positive Schistosomiasis Status

Variable	Crude OR	P-Values
Sex (female)	0.99 (0.43, 2.30)	0.9873
Incoming Discordant	1.89 (0.81, 4.39)	0.1416
Age<=30 years	1.35 (0.58, 3.13)	0.4836
>15 Years in Lusaka	2.38 (0.87, 6.47)	**0.0901**
HIV Positive Serostatus	1.73 (0.68, 4.40)	0.2477
Positive Ascaris lumbricoides Infection	6.24 (1.66, 23.41)	**0.0067**
Low Positive ELISA Titer (>26.3 and <52.7)	2.30 (0.82, 6.42)	0.1124
High Positive ELISA Titer (>=52.7)	1.31 (0.44, 3.92)	0.6262
Positive Western Immunoblot Result for S. haematobium	1.52 (0.57, 4.02)	0.4026
Positive Western Immunoblot Result for S. mansoni	0.46 (0.12, 1.69)	0.2403
Childhood Swimming and Bathing Exposure	2.11 (0.67, 6.65)	0.2019
Childhood Water Source Used by Community for Urination and Defecation	1.01 (0.11, 9.09)	0.9932
Currently Lives in Lusaka Central	1.38 (0.57, 3.32)	0.4727
Unemployed	1.57 (0.64, 3.87)	0.3261
Lived in Village or Rural Area before Age 16	0.69 (0.28, 1.72)	0.4258
Little or No English Literacy	0.50 (0.20, 1.25)	0.1404
Erectile Dysfunction (Men (n=13)	3.25 (0.70, 15.1)	0.1330
Painful Ejaculation (Men (n=13)	1.40 (0.14, 13.6)	0.7737
Nyanja Tribe	1.85 (0.75, 4.58)	0.1815
Frequent Urination	0.42 (0.05, 3.61)	0.4306
Stress Incontinence (Women (n=12)	1.18 (0.22, 6.29)	0.8481
Usage of Intravaginal Herbs (Women(n=12)	0.63 (0.12, 3.19)	0.5731
Infertility	4.40 (0.92, 21.01)	**0.0634**
Bloody Urine	1.07 (0.29, 4.02)	0.9170

Bold=Significant p-value<0.10

Table 14: LOGISTIC MODEL

Variable	Adjusted OR (CI)	Chi-Square	p-value
Age<=30	7.8 (1.9, 31.1)	8.40	0.0038
ELISA>=52.68	5.3 (1.1, 26.1)	4.13	0.0420
Childhood Water Exposure	3.2 (0.7, 15.6)	2.06	0.1509
Positive Western Blot for S. haematobium	0.83 (0.21, 3.22)	0.08	0.7829
>15 Years in Lusaka	4.0 (1.00, 16.5)	3.80	0.0515
Ascaris lumbricoides Positive	10.6 (1.1, 103.2)	4.12	0.0424

Table 15: Model Fit Statistics

R-Square	0.3285
Hosmer and Lemeshow Goodness of Fit Test	p-value=0.6340
Likelihood Ratio	0.0060
AUROC	0.8019

APPENDIX 1: Site-Specific Protocol

Rwanda Zambia HIV Research Group

Schistosomiasis Detection with Urine Filtration and Kato-Katz Fecal Smears
Site Specific Procedure (Adapted from WHO 2008)

Schistosomiasis Detection with Urine Filtration and Kato-Katz Fecal Smears	
Revision Date: 30 June 2011	**Effective Date:**
Expiry Date:	**Retired Date:**
Lab SSP: 3	**Version: 1.0**

Authorized by Principal Investigators and/or their designee:

_____	_____	_____
Printed Name, Title	Signature	Date
_____	_____	_____
Printed Name, Title	Signature	Date

Name of study staff who have read and understand this Standard Operating Procedure	**Signature**	**Date**

1. PURPOSE

To describe the procedures used for Millipore© Urine Filtration and Kato-Katz Fecal Kits to test for schistosoma ova in urine and fecal samples. To ensure that tests are being performed according to the manufacturer's recommendations and safety guidelines, and that quality control procedures are adhered to in order to provide accurate results.

2. DEFINITIONS

None

3. RESPONSIBLE PARTIES

3.1. Laboratory technicians, who are suitably trained and competent, will assist the student in performing tests and summarizing results

3.2. The laboratory manager or intern will perform internal quality control once the test is completed.

4. PROCEDURES

4.1 Millipore Urine Filtration

4.1.1 Principle:

- Urine sample is passed through a biological filter containing pores of a specific diameter (Millipore). This condenses the elements (cellular and amorphous) of the urine in a small surface in order to obtain good quality preparations for microscopy.

4.1.2 Warnings and Precautions:

- Handle samples as potentially infectious and always wear the appropriate personal protective equipment. To review procedures for health and safety in the lab, refer to Lab SOP 1B Health and Biosafety in Laboratory.

4.1.3 Materials and supplies:

- Lugol's Iodine
- Universal container (50ml)
- Microscope
- ACD tubes
- Syringe (10ml)
- Disinfectant
- Filter/Filter unit holder
- Microscope slides

4.1.4 Specimen Collection and Storage:

- Urine:May be stored at 2-8 ^{o}C for up to 24 to 48 hours
- Blood Plasma: When collecting routine ACD plasma, aliquot 50µl in a schistosomiasis antibody box to be shipped to CDC/Emory. Once aliquotted specimens will be stored in -80.

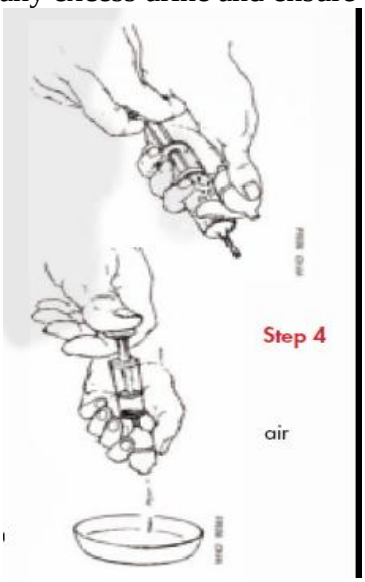
4.5 Assay Procedures:

4.5.1 Unscrew the Filter Holder and Carefully Place One Filter inside the holder, making sure it fits correctly.

4.5.2Shake and mix the urine sample before drawing a 10ml sample into a syringe. Attach the filter unit.

4.5.3 Keeping the syringe and unit in vertical position, press plunger down to push all the urine through the filter and out into a bucket/liquid waste receptacle with 10% disinfectant added to receptacle (bleach is ok).

4.5.4 Carefully detach the syringe from the filter unit. Draw air into the syringe, reattach the syringe to the filter unit holder and expel the air again. This is important to remove any excess urine and ensure the eggs are firmly attached to the filter.



4.5.5 Unscrew the unit and remove the filter, placing it (top side up) onto a microscope slide.

4.5.6 Add one drop of Lugol's Iodine and wait 15 seconds for the stain to penetrate the eggs. This makes the eggs more visible.

4.5.7 Immediately examine the whole filter under a microscope at low power (x40). Schistosome eggs can be seen clearly as they will stain orange. Infection loads are recorded as number of eggs as viewed in entire field per 10ml of urine.
4.5.8 Record all results in the Schisto Lab log book (Appendix 1)

4.2 Kato-Katz Fecal Smear

4.2.1 Principle:

- Glycerol is used to clarify the thick smear preparation. This makes the eggs easier to visualize under the microscope which increases the accuracy of diagnosis under field conditions.

4.2.2 Warnings and Precautions:

- Handle samples as potentially infectious and always wear the appropriate personal protective equipment. To review procedures for health and safety in the lab, refer to Lab SOP 1B Health and Biosafety in Laboratory.

4.2.3 Materials and supplies:

- Nylon screen
- Cellophane
- Glycerol
- Distilled water
- Stool collection container
- Newspaper/paper/tissue paper
- Spatula
- Microscope slides
- Microscope
- Flat bottom jar

4.2.4 Specimen Collection and Storage:

- Feces: May be stored at 2-8 ^{o}C for up to 24 hours.
- Blood Plasma: When collecting routine ACD plasma, aliquot 50µl in a schistosomiasis antibody box to be shipped to CDC/Emory per visit (repeated from section 4.5.4 above, collection only done once. Once aliquotted specimens will be stored in -80.

4.2.5. Test Procedures

1. The day before testing (at least 24 hours), cut number of pieces of nylon screen you will need, cut number of pieces of cellophane you will need, place cellophane strips in jar.
2. Pour 100ml glycerol and 100ml distilled water into the jar; leave overnight
3. When fecal sample is brought, place small amount of feces on newspaper or scrap paper and press piece of nylon screen on top so that some of feces are sieved through screen and accumulated on top
4. Scrap the flat-sided spatula across the upper surface to collect the sieved feces
5. Place template on center of microscope slide and add feces from spatula so that hole is completely filled
6. Pass over the template using the side of the spatula to remove excess feces from the edge of the hole (spatula and template may be discarded or reused if carefully washed)
7. Remove the template carefully so that cylinder of feces is left on slide
8. Cover fecal material with pre-soaked cellophane strip. Strip must be very wet if feces are dry and less wet if feces are soft (if excess glycerol solution is present on surface of cellophane, wipe with toilet paper).
9. Invert microscope slide and press fecal sample firmly against cellophane strip (hydrophilic) on another microscope slide or on smooth hard surface. Fecal material should be spread evenly between microscope slide and cellophane strip. It should be possible to read newspaper print through smear after clarification
10. Carefully remove slide by gently sliding sideways to avoid separating cellophane strip. Place slide on bench with cellophane upwards. Water evaporates while glycerol clears the feces
11. Keep slide for one or more hours at room temperature to clear fecal material prior to microscope examination. To speed up clearing, slide can be placed in direct sunlight for several minutes or placed in an incubator 40C.
12. Examine slide within 24 hours in systematic manner and noting number of eggs of each species (see bench aids). Multiply this number by 24 to obtain the number of eggs per gram of feces (epg).
13. Egg gives indication of schistosoma parasite burden and intensity of infection
    a. 1-99epg=Light Infection
    b. 100-399epg=Moderate Infection
    c. >400epg=Heavy Infection

4.2.6 Result Reporting

1. Record results for both the Urine Filtration and the Kato Katz smear in the Schisto Lab log book (Appendix A.)
2. Lab Result Slips: record the result on the lab result slip which should be QC'd by intern/manager or supervisor.
3. In case of discrepant result (Urine and Fecal tests do NOT yield same result), choose fecal smear as correct result to report to clinic.
4. The Schisto Lab Log will record both results for the urine and fecal smear even if results are discrepant (i.e. Urine positive and Fecal Negative or vice versa).

EXAMPLE:

HTID: 3772 M
Schisto: +
Initials: PDL

5.1 Appendix I: Schisto Lab Log

HTID	Sex	Month	VisitDate	Age	Group-95 Follow Up (FU) or Incoming Discords (ID)	Ova in Urine (Y/N)-Number (epg)	Ova in Fecal Smear (Y/N) – Number (epg)	HIV Status (+/-/D)	Genital Symptoms (please specify)

Appendix 2: Informed Consent

Bilharzia is transmitted by tiny parasites which live in snails that live in rivers, lakes and ponds. The parasites are expelled by the snails out into the water, where especially if the water is not free-flowing, can infect any person washing, bathing or swimming there. When the parasite gets inside you, it spreads out its eggs which try to leave the body and lodge in the bladder, reproductive system and the rectum. Most people are infected as children but this can cause a problem in adulthood because the eggs create bleeding and lesions that might let HIV pass through from person to person. However, bilharzia cannot be sexually transmitted.

We are carrying out research to understand how many people that come to ZEHRP have bilharzia and what aspects of your life may put you at risk for getting this disease. As many people who have bilharzia may not have symptoms, we will need to collect one urine sample and one fecal sample to assess your bilharzia status. These are cheap and effective ways to see if a person has the disease. Bilharzia has also been linked to increased risk of sexually transmitted infections, vaginal pain and bleeding and infertility in women. In men, it has been linked to erectile dysfunction, infertility and pain during ejaculation. Treatment is available, if you are found to have bilharzia infection, and will be provided to you at no charge. These samples will allow us to inform you of your bilharzia status and help us give you treatment, if you need it.

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