HIV and Schistosoma spp. interactions: epidemiology and consequences for detection and prevention in the lake region of Tanzania

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ABBREVIATIONS

Ab	Antibody
Adj	Adjusted
Ag	Antigen
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
ART	Antiretroviral Therapy
ARV	Antiretrovirals
BMC	Bugando Medical Centre
CAA	Circulating Anodic Antigen
CB	Community-Based
CCA	Circulating Cathodic Antigen
CCR	C-C Chemokine Receptor
CD4	CD4 T+ Lymphocytes
CI	Confidence Interval
CTC	Care and Treatment Clinic
CXCR	C-X-C Chemokine Receptor
DALY	Disability-Adjusted Life Years
DBS	Dried Blood Spot
DSS	Demographic Surveillance System
ELISA	Enzyme-Linked Immunosorbent Assay
HCT	HIV Counseling and Testing
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IFN	Interferon
Ig	Immunoglobuline
IL	Interleukine
IQR	Interquartile Range
IV	Intra-Venous
ln	Natural Logarithm
log10	Logarithm 10
MDA	Mass Drug Administration
n	Numerator
N	Total Denominator
NA	Not Available or Not Applicable
NIMR	National Institute for Medical Research
OB	Outpatient clinic-Based
OR	Odds Ratio
p	P-value
PCR	Polymerase Chain Reaction
PLHIV	People Living with HIV

POC	Point Of Care
PRR	Prevalence Risk Ratio
PZQ	Praziquantel
Ref	Reference
RNA	Ribonucleic Acid
Schisto	*Schistosoma* infection
Sh* or *S.h.	*S. haematobium*
sHIV	simian HIV
SHR	Subdistribution Hazard Ratio
Sm* or *S.m.	*S. mansoni*
STI	Sexually Transmissible Infection
TB	Tuberculosis
TCA	Trichloroacetic Acid
Th	T-helper cell
TNF	Tumor Necrosis Factor
T reg	Regulatory T cell
UCP-LF	Luminescent Up-Converting Phosphor Lateral Flow
UNAIDS	Joint United Nations Programme on HIV and AIDS
Un.	Unidentified
VCT	Voluntary Counseling and Testing
VL	Viral Load
WASH	Water, Sanitation, and Hygiene
WHO	World Health Organization
yo	Years-old

CHAPTER 1 – GENERAL INTRODUCTION

I. CONTEXT FOR THIS THESIS

*1) Epidemiology of HIV/*Schistosoma *spp. co-infections and study site*

Approximately 36.7 million people are infected with HIV around the world while 218 million are infected with *Schistosoma* spp.[1, 2]. These infections overlap and an estimated 6 million individuals are HIV/*Schistosoma* spp. co-infected[3-5]. The majority of co-infections occur in Africa[4].

The coastline of Lake Victoria in East Africa too harbors both diseases[1, 2] and, due to common risk factors, such as occupation and socio-economic status[6, 7], is a site with a high level of co-infections. We have set our studies in the Lake Zone of Tanzania, the Tanzanian side of Lake Victoria. It comprises 6 regions and has a population of about 11.8 million inhabitants, representing one-fourth of the country's population[8, 9]. The Lake Zone is predominantly rural, with fast changing demographics regarding urbanization and the increased availability of healthcare[8, 9].

In East Africa, both *S. haematobium* and *S. mansoni* are found, with prevalences ranging from 1% to over 80% in the Lake Zone of Tanzania[10, 11]. The HIV prevalence in the Lake Zone of Tanzania varies between 3.6% and 7.4%[12]. In the context of the HIV/AIDS epidemic, co-infections typically exacerbate morbidity and mortality, as shown by studies looking at interactions between HIV and tuberculosis, cryptococcosis, hepatitis B virus, hepatitis C virus, and malaria[13, 14]. The immunodeficiency caused by chronic HIV infection increases the risk of co-infection with many pathogens[13, 14]. Moreover, administration of antiretroviral therapy (ART) does not always restore the pathogen-specific immune response to co-infections to normal levels[14]. We might thus expect HIV infection to increase morbidity associated with *Schistosoma* spp., and likely *vice versa*.

This thesis seeks to add to the current knowledge on HIV and *Schistosoma* spp. co-infections through new research questions and identification of gaps in the current methodology.

2) Genital schistosomiasis and its pathologies

S. haematobium most prominently affects the genitourinary system while *S. mansoni* affects the gastro-intestinal system[15]. Clinical manifestations of schistosomiasis are caused by the eggs laid by adult worms of the *Schistosoma* spp. that live in the vasculature. Eggs secrete proteolytic enzymes as they migrate through tissues en route to the lumen of the urinary bladder or intestine, where they are subsequently excreted in urine or stool[16]. When the eggs are sequestered in the uro-genital tract, the disease caused is called urogenital schistosomiasis, which is marked by inflammation, friability, and bleeding of the urinary and genital mucosa[17, 18]. Late-stage complications of urogenital schistosomiasis include bladder cancer and urinary tract obstruction with hydronephrosis[17, 18]. *S. mansoni* eggs can also be found in the urogenital tract, resulting in both species being able to cause urogenital schistosomiasis[19,20]. It is estimated that *S. mansoni* causes 1 case of urogenital

schistosomiasis for every 4 cases of urogenital schistosomiasis caused by *S. haematobium*[19, 20]. *S. mansoni* eggs most frequently cause disease in the lower intestine and the liver, leading to bloody diarrhea and liver periportal fibrosis. Advanced *S. mansoni* infection can lead to ascites, variceal hemorrhage, and death[15]. Differences in clinical diseases, diverse physical and immunologic effects of eggs in different tissues, and host characteristics all contribute to the observed differences in interactions between *Schistosoma* and HIV infections[19, 20].

Men and women are affected differently by genital schistosomiasis. In men, eggs are found at the highest frequency in the seminal vesicles with high egg per gram counts. Eggs are also found in the prostate, testes, and epididymis, thus affecting mostly internal organs[17, 20-22]. Genital lesions due to eggs sequestration and induced inflammation are less common in men than in women[17, 21, 23], and the burden of infection in men is not always proportional to the degree of inflammation [21]. In women, eggs are found both in the cervix and the vagina at high frequency, with high counts of eggs per gram in the vagina[22, 23]. Eggs can also sometimes be found in the ovaries, fallopian tubes, and uterus[23]. Sequestered eggs and their associated lesions in women are thus found in sites directly accessible during sexual contact[18].

Schistosoma eggs are highly immunogenic and lead to recruitment of immune cells including Th2 helper cells and macrophages that are preferential targets of HIV. Several studies on urogenital schistosomiasis in women found a variety of tissue reactions surrounding the ova associated with higher densities of genital mucosal CD4+ T lymphocytes (CD4), macrophages, and eosinophils compared with cervicovaginal mucosa without ova[24]. The cell modifications shown in women are likely to be similar in men[17]. In addition, increased levels of leukocytes as well as expression of IL-4, IL-6, IL-10, IFN-γ, and TNF-α have been found in semen of *S. haematobium*-positive men, which in return might induce accelerated replication of HIV[25].

3) Diagnosis of HIV and Schistosoma *spp. infection*

The Tanzanian national guidelines for HIV testing follow WHO recommendations. The standard procedure for diagnosis of HIV in our study population involves the use of rapid tests for antibody testing[26]. A positive screening test is followed by a second and different confirmatory rapid test. To determine when to initiate ART and monitor response to treatment and progression of the disease, CD4 counts were measured every 6 months until 2016[26]. Since 2016, monitoring guidelines changed as HIV RNA viral load quantification using PCR was implemented[27]. Before and at start of ART, CD4 counts are still measured, but anyone with HIV is started on ART regardless of their CD4 counts. After ART initiation, HIV RNA viral load testing is used to evaluate response to ART, with a first HIV RNA viral load test 6 months after initation. Routine testing is then implemented every 12 months if the patient is virally suppressed or every 3 months if the patient is not responding to treatment[27]. Where HIV RNA viral load monitoring is unavailable, clinical monitoring and CD4 monitoring are still in use[27]. Since 2016, HIV RNA viral load testing in the Lake Zone is performed at Bugando Medical Centre, the zonal referral hospital serving 15 million people in the Lake Zone. However, with small clinics progressively sending their samples for HIV

RNA testing further upscaling of HIV RNA viral load testing is needed to prevent back-log. Very little data on HIV RNA viral load was available before 2016. The progress in HIV testing and access to health care is described in **Chapter 2**.

Both CD4 counts and HIV RNA viral load change over the course of a natural HIV infection, and are predictors of the course of the HIV infection[28-30]. They vary proportionally as a higher HIV RNA viral load leads to a lower CD4 count due to the pathogenesis of the virus. The natural course of an HIV infection can be divided into three stages, all characterized by different slopes of decline/increase in CD4 counts and HIV RNA viral load: the primary infection, a latent stage, and AIDS[31]. For CD4 counts, significant declines in the systemic levels of CD4 cells occur in the first 2–8 weeks following HIV-1 infection[32]. CD4 counts then re-increase slightly to progressively decline again with an average yearly loss of approximately 60 CD4 cells/μl[33], (varying according to HIV type), during the latent stage of the disease. Over a period of years the decrease in CD4 counts leads to death from immune failure and opportunistic infection[34, 35]. After 8-12 years, without ART initiation, CD4 counts drop below 200 cells/μl making the patient susceptible to AIDS-defining opportunistic infections and neoplasms[36, 37]. The decline in the level of CD4 typically continues until reaching the null, but the decline is not seen in the small percentage of individuals who are long-term nonprogressors. Primary infection is also accompanied by a burst in HIV RNA viral load, mirroring viral replication[38-40]. Antiviral immune responses further lead to high declines in plasma viremia [41-43], which then stabilize. This steady-state HIV RNA viral load is called the viral load set point and is highly predictive of HIV transmission and progression to AIDS disease[44]. There is continuous viral replication during the latent stage as it is a clinical latency rather than a viral latency[45, 46]. At the end stage of the infection, HIV RNA viral loads peak again to virtually infinity. Disease progression is directly linked to HIV RNA viral load and to the extent of viral replication[44].

In the absence of ART, the natural course of HIV infection can vary widely with some HIV-positive individuals able to maintain high CD4 counts and/or suppressed HIV RNA viral load.

The current gold standard for diagnosis of active *Schistosoma* infection and detection of the species, as recommended by the WHO, is microscopy on urine or stool [2], via urine filtration or Kato Katz technique for stool. It is well recognized that egg excretion varies, depending on prevalence, time of the day, and worm burden[47-53], and that microscopy may be less sensitive than other diagnostic strategies including antigen testing and PCR[54]. In addition, it is tedious, needs an experienced microscopist, and ideally would require multiple sampling. An advantage of microscopy is the near-perfect specificity and the ability to differentiate between *Schistosoma* species, which is not possible with some of the other diagnostics.

Although antibody-based tests exist and are cheap and easy to use for *Schistosoma* spp. testing[54], they do not allow differentiation of active versus past *Schistosoma* spp. infection and are therefore not often used in endemic settings. They do have a role in diagnosis of travelers who would not be expected to have been previously exposed to *Schistosoma* spp.

infection. Current PCR techniques detect active infection but are expensive and often do not distinguish between *Schistosoma* species[54, 55]. In addition, some of the PCRs described remain positive for a long time after clearance of the infection due to slow degradation of the sequestered eggs[56].

Schistosome antigen testing is increasingly accepted as a highly sensitive technique for diagnosis of *Schistosoma* spp., although it does not allow distinction between *Schistosoma* species[54]. Circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) are glycosaminoglycan-like carbohydrates produced in the gut of adult schistosome worms and regurgitated into the host bloodstream during active infection with any *Schistosoma* species (including veterinarian)[57]. They can be detected in the serum and urine of infected individuals, and the level of these antigens is proportional to the intensity of infection[54]. CAA and CCA levels rise and fall rapidly post-infection and post-treatment respectively[58-60, 61] and do not seem to present any circadian variability[61]. The CAA and CCA based tests have been optimized for improved sensitivity and use in the field and each have their specific application. User-friendliness and sensitivity of the CAA assay was initially improved utilizing the luminescent up-converting phosphor technology in combination with a lateral flow-based platform (UCP-LF)[62] as well as the introduction of a sample concentration method[63,64]. This genus specific test requires some basic laboratory equipment and includes a sample preparation step with incubation that permits sample concentration and ultimate sensitivity (single worm detection)[63, 64]. It can be used on blood-derived samples as well as urine and saliva. The UCP-LF assay for CAA detection has been adapted to a dry reagent format that allows convenient storage at ambient temperature and shipping without the need for a cold chain[65], and alternative methods are explored to make the sample preparation and concentration step more field applicable[66]. As CAA is extremely stable[52], this assay can also be applied on dried blood spot samples[67, 68]. For CCA detection, a commercial lateral flow based assay for rapid point-of-care testing on urine is available, POC-CCA (Rapid Medical Diagnostics, Pretoria, South-Africa). The test allows rapid identification of active infections, and is recommended for medium to high endemic *S. mansoni* settings[69].

II. *SCHISTOSOMA* AND HIV CO-INFECTIONS: GAPS IN KNOWLEDGE

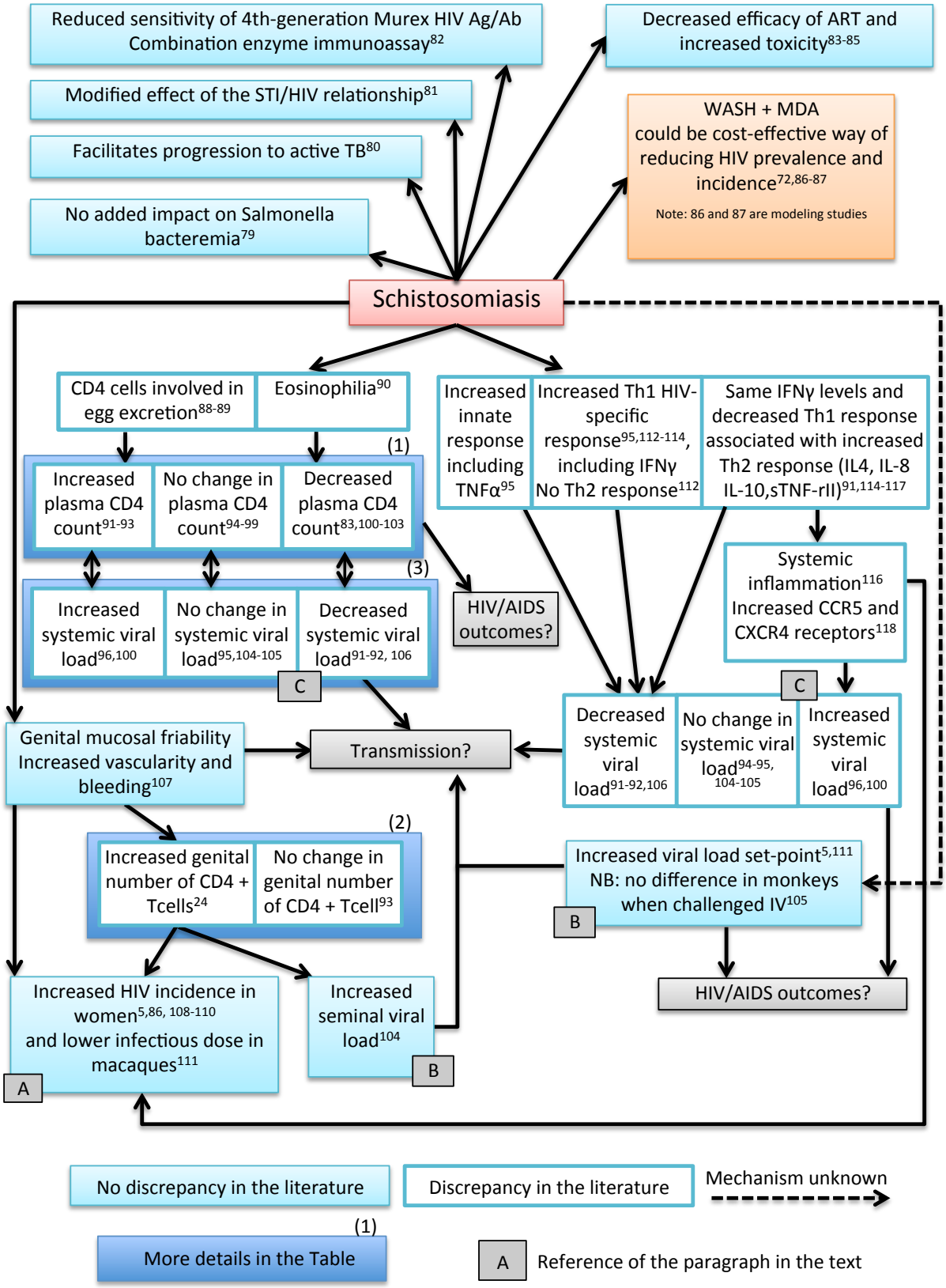
Despite multiple studies reporting interactions between infection with HIV and *Schistosoma* spp. in humans and macaques, little is still known about the processes and modalities of this co-infection. A systematic search on Pubmed for all scientific publications focusing on the epidemiology, immunology, and clinical aspect of HIV/*Schistosoma* spp. co-infections yielded only a total of 85 publications from 1985 to 2017. Of these, 10 were literature reviews, and 6 were case reports.

Several cross-sectional studies and mathematical models have reported a clear association between HIV and *Schistosoma* spp. infection in women but not in men[4, 70-78]. Other studies have explored specific directions of the relationship between HIV and *Schistosoma* spp., looking separately on the one hand at the effect of *Schistosoma* spp. on HIV susceptibility and disease, and on the other hand at the effect of HIV on *Schistosoma* spp. infection. The knowledge and gaps (at the start of the investigations presented in this thesis) on the epidemiology of HIV/*Schistosoma* spp. co-infections, and the immunological and mechanical processes behind it are summarized below.

1) Effect of Schistosoma *spp. infection on HIV susceptibility and disease*

The effect of *Schistosoma* spp. on HIV susceptibility and disease is described in **Figure 1**. Each arrow describes a plausible or reported causal pathway, starting from co-infection with *Schistosoma* spp. all the way to HIV susceptibility and disease. The pale blue boxes show findings for which either only one study was conducted, or for which there is agreement in the literature. The white boxes show discrepant findings. The parts of the graph indicated with capital letters are described in more details below. The dark blue boxes are detailed in **Table 1** below.

Figure 1 - Co-infection conceptual framework – Effect of Schistosoma spp. infection on HIV susceptibility and disease progression.



A. Effect of *Schistosoma* spp. on HIV susceptibility

One longitudinal study and several mathematical models indicated that *Schistosoma* spp. infection is a risk factor for HIV acquisition in women, but not in men[5, 86, 108, 109]. In addition, in macaques infected with *Schistosoma mansoni*, lower doses of simian HIV (sHIV) are required for infection[111]. Research on co-infections has so far been lacking longitudinal studies to confirm the relationship and define a link of causality. Only two longitudinal studies investigated the link of causality between *Schistosoma* spp. infection and HIV acquisition in humans [5,110]. One did not find any[110], while the other found a link of causality only when stratifying by sex[5]. Longitudinal studies thus need to be repeated and results should be confirmed in other settings for both species of *Schistosoma*.

B. Effect of *Schistosoma* spp. on HIV RNA viral load

In addition to demonstrating increased HIV susceptibility, one of the longitudinal studies in Tanzania also found increased HIV RNA viral load set-points in both men and women who were infected with *Schistosoma* spp. at time of HIV seroconversion[5]. *Schistosoma* spp. co-infection has also been associated with increased local seminal HIV RNA viral load[104] and increased systemic HIV RNA viral load in some studies[96, 100]. A similar effect of *S. mansoni* infection was also observed in macaque studies of sHIV[105].

If *Schistosoma* spp. co-infection impacts systemic and local seminal HIV RNA viral loads, then we would expect to see worse HIV-AIDS outcomes in co-infected individuals given the known worse outcomes with higher HIV plasma RNA viral loads[119]. Furthermore, we would expect an increased transmission to sexual partners (independent of the sexual partner *Schistosoma* spp. status)[119, 120]. Only one study, to our knowledge, has investigated the impact of *Schistosoma* spp. infections on HIV-related outcomes. This randomized trial looked at the effect of annual praziquantel (PZQ) treatment (25 mg/kg) on CD4 counts and death[97]. Treatment was given empirically, regardless of the infection status, and baseline estimate of prevalence of *Schistosoma* spp. in the treatment and control group were unknown. This study led to inconclusive results likely due to empiricality of treatment and the low prevalence of *S. mansoni* (about 2%) previously reported in the area[97, 121].

This thesis investigates the relationship between *Schistosoma* spp. infection at time of HIV seroconversion and HIV/AIDS outcomes as defined by death and CD4 counts in **Chapter 3**. We hypothesized that *Schistosoma* spp. infection at time of HIV seroconversion would lead to worse HIV-AIDS outcomes. In **Chapter 4**, we quantify the impact of *Schistosoma* spp. infection in HIV positive individuals on intra-marital transmission of HIV to a serodiscordant spouse. We hypothesized that *Schistosoma* spp. co-infection would lead to increased HIV-1 transmission to serodiscordant spouse. Both were retrospective longitudinal studies that used stored Dried Blood Spots (DBS) for the testing of *Schistosoma* spp..

C. Discrepancies in the results

There is discrepancy in the literature regarding the effect of *Schistosoma* spp. infection on systemic HIV RNA viral load and CD4 counts[5, 24, 83, 91-98, 100-104, 106], with studies documenting either higher or lower HIV RNA viral loads/CD4 counts, or no effects[5, 91, 92, 94-97, 100, 104, 106]. Most studies have studied the effect of *Schistosoma* spp. infection on systemic HIV RNA viral load or CD4 counts by treating co-infected individuals with PZQ and comparing baseline HIV RNA viral load and CD4 counts to post-treatment values[91, 92, 94, 97, 100, 104, 106]. The length between treatment and re-testing varies, leading to contradicting results, and studies showing transitory increase in HIV RNA viral load and decrease in CD4 counts after treatment[92, 94, 100, 106].

HIV RNA viral load and CD4 counts are markers of the virological and immunological aspects of the HIV/*Schistosoma* spp. relationship and tools for understanding HIV disease progression. Efforts to understand the reason behind those differences in reported HIV RNA viral loads and CD4 counts in relationship to *Schistosoma* spp. infections are crucial to define the interactions between HIV and *Schistosoma* spp. In the meantime, establishing uniform and replicable study designs and analysis methods would minimize confounding and allow comparability of the studies. In **Table 1**, we present the studies with conflicting results to highlight the differences and potential reasons for the findings. Duration of HIV infection, duration of *Schistosoma* spp. infection, duration of co-infection, age, sex, and ART intake are all potential confounders and effect modifiers[5, 28-30, 122-124] that are rarely studied in depth when looking at the epidemiology of HIV/*Schistosoma* spp. co-infections.

In **Chapter 5**, we seek to find an explanation to the discrepancies found regarding the effects of *Schistosoma* spp. on systemic HIV RNA viral load. We hypothesized that duration of HIV infection, which largely affects HIV RNA viral load[28-30] could have been a main confounder in past studies.

Table 1 - Relationship between *Schistosoma* infection and CD4 counts and HIV RNA viral loads.

Reference	Total sample size (% schisto)	Study type	Species	*Schistosoma* test	Population	ART	Findings in those with *Schistosoma* co-infections
(1) Plasma CD4 count							
91. Brown M et al., 2005. J Infect Dis	152 (100%)	-Longitudinal -Treatment with PZQ -3 follow ups, up to 5 months	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART not available at time of study	Increased plasma CD4 count
92. Elliott AM et al., 2003. Trans R Soc Trop Med Hyg	108 (26%)	-Longitudinal -Treatment with PZQ -2 follow-ups up to 4 months	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART Naive	Increased plasma CD4 count
93. Prodger JL et al., 2015. PLoS Negl Trop Dis	24 (50%)	Cross-sectional	*Sm*	Kato-Katz Urine-CCA	Human Adults Male only	NA	Increased plasma CD4 count
101. Kallestrup P et al.,2005. J Infect Dis	356 (75%)	Cross-sectional	All	Microscopy CAA	Human Adults Male and Female	ART not widely available at time of study	Decreased plasma CD4 count for *S. mansoni* No change in plasma CD4 count for *S. haematobium*
94. Brown M et al., 2004. J Infect Dis	401 (42.9%)	-Longitudinal -Treatment with PZQ -Only 1 follow-up at 6 months	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART naive	No change in plasma CD4 count
95. Obuku AE et al., 2016. AIDS Res Hum Retroviruses	34 (52.9%)	Cross-sectional	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART naive	No change in plasma CD4 count
97. Walson J et al., 2012. Lancet Infect Dis	877 (2%)	-Longitudinal study -Treatment with PZQ -Follow-up every 3 months, up to 24 months	All	Kato-Katz PCR	Human Adults Male and Female	Eligible participants did not meet criteria for ART initiation	No change in plasma CD4 count

98. Noormahomed EV et al., 2014. PLoS Negl Trop Dis	601 (23%)	Cross-sectional	All	Western Blot	Human Adults Male and Female	Taken into account as a confounder	No change in plasma CD4 count
100. Kallestrup P et al., 2005. J Infect Dis	227 (100%)	-Longitudinal -Treatment with PZQ -Only 1 follow-up at 3 months	All	Microscopy CAA	Human Adults Male and Female	ART not widely available at time of study	Decreased plasma CD4 count
83. Efraim L et al., 2013. J Acquir Immune Defic Syndr.	351 (27.6%)	Cross-sectional	All	Microscopy Urine CCA	Human Adults Male and Female	Immunologically failing on ART	Decreased plasma CD4 count
102. Mwinzi PN et al., 2004. Am J Trop Med Hyg.	81 (100%)	Cross-sectional	*Sm*	Kato Katz Liver ultrasound	Human Adults Male only	NA	Decreased plasma CD4 count
103. Sadlier CM et al., 2013. AIDS Res Ther	90 (7.7%)	Cross-sectional	All	ELISA	Human Adults Male and Female	NA	Decreased plasma CD4 count
(2) CD4 counts in genital tissue							
24. Jourdan PM et al., 2011. Am J Trop Med Hyg	61 (100%)	Cross-sectional	*Sh*	Microscopy	Human 15-49 yo Female only	NA	Increased number of CD4 count in cervical mucosa
93. Prodger JL et al., 2015. PLoS Negl Trop Dis	24 (50%)	Cross-sectional	*Sm*	Kato-Katz Urine-CCA	Human Adults Male only	NA	No change in number of CD4 count in penile foreskins
(3) Systemic HIV RNA viral load							
100. Kallestrup P et al., 2005. J Infect Dis	227 (100%)	-Longitudinal -Treatment with PZQ -Only 1 follow-up at 3 month	All	Microscopy CAA	Human Adults Male and Female	ART not widely available at time of study	Increased systemic HIV RNA viral load

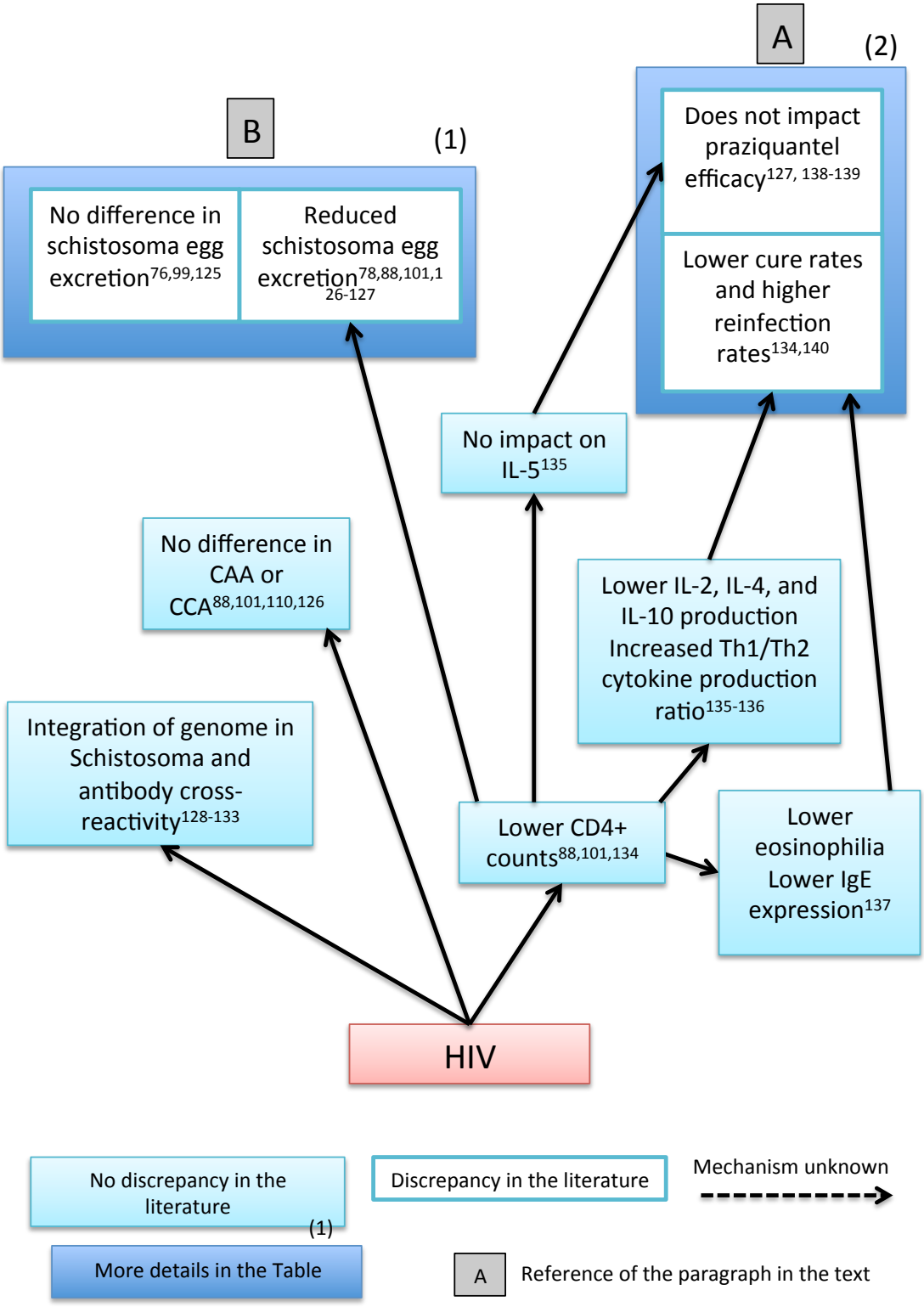
96. Sangare LR et al., 2011. Parasitology	4 articles	-Systematic literature review and meta-analysis	*Sm*	NA	Human Adults Male and Female	NA	Increased systemic HIV RNA viral load
104. Midzi N et al., 2017. Open Forum Infect Dis	18 (100%)	-Longitudinal -Treatment with PZQ -Only one follow-up	*Sh*	Microscopy	Human Adults Male only	Results stratified by ART	No change in systemic HIV RNA viral load
94. Brown M et al., 2004. J Infect Dis	418 (39.0%)	-Longitudinal -Treatment with PZQ -Only one follow-up at 6 months	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART naive	No change in systemic HIV RNA viral load
95. Obuku AE et al., 2016. AIDS Res Hum Retroviruses	34 (52.9%)	Cross-sectional	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART naive	No change in systemic HIV RNA viral load
105. Siddappa NB et al., 2011. PLoS Negl Trop Dis	15 (53.3%)	-Longitudinal -Follow ups, up to 20 weeks	*Sm*	NA	Macaques Adults Female only	NA	No change in systemic HIV RNA viral load
91. Brown M et al., 2005. J Infect Dis	119 (100%)	-Longitudinal -Treatment with PZQ -3 Follow ups, up to 5 months	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART not available at time of study	Decreased systemic HIV RNA viral load
92. Elliott AM et al., 2003. Trans R Soc Trop Med Hyg	108 (26%)	-Longitudinal -Treatment with PZQ -2 follow-ups up to 4 months	*Sm*	Kato-Katz CAA	Human Adults Male and Female	ART naive	Decreased systemic HIV RNA viral load
106. Lawn SD et al., 2000. AIDS	30 (100%)	-Longitudinal -Treatment with PZQ -Only one follow-up	*Sm*	Kato-Katz Plasma CCA	Human Adults Male only	NA	Decreased systemic HIV RNA viral load

schisto = *Schistosoma*
Sm = *S. mansoni*
Sh =*S. haematobium*
NA = Not available or not applicable

2) Effect of HIV on **Schistosoma** *spp. infection*

The effect of HIV on *Schistosoma* spp. infection is described in **Figure 2**. Each arrow describes a plausible or reported causal pathway. The diagram focuses on two aspects of the impact of HIV infection on *Schistosoma* spp.: section A illustrates the impact on PZQ efficacy and section B shows the impact on egg excretion. The pale blue boxes show findings for which either only one study was conducted, or for which there is agreement in the literature. The white boxes show discrepand findings. The parts of the graph indicated with capital letters are described in more details below. The dark blue boxes are detailed in **Table 2** below, providing additional information regarding several discrepant studies.

Figure 2 - Co-infection conceptual framework – Effect of HIV on *Schistosoma* spp. infection.



A. Effect of HIV on praziquantel efficacy

The role of HIV positivity on the efficacy of PZQ is unclear. Some studies have reported lower cure rates and higher rates of reinfection as measured by microscopy testing (egg detection)[134, 140] also in association with decreased immunity against *Schistosoma*[135-137]. Others have not been able to detect any effect[127, 138, 139]. PZQ treatment usually increases the Th2:Th1 ratio and the level of adult worm specific interleukins, including IL-2, IL-4, IL-5, and IL-10, leading to killing of adult worms and low chance of reinfection[141-144]. In addition, high levels of eosinophilia and of IgE expression are related to resistance against re-infection with schistosomes[142, 145, 146]. Contradictory, for HIV+ individuals, studies have shown that after treatment more Th1 cytokines are produced and IL-2, IL-4 and IL-10 production is decreased[135, 136]. This could explain the lower cure rates and higher reinfection rates seen in studies on co-infected individuals[134, 140]. CD4 declines in HIV+ individuals also lead to decreased ability to develop eosinophilia[137], and lower levels of IgE expression have also been observed[137], providing an additional (or alternative) explanation for the higher rates of reinfection with *Schistosoma* spp. observed in HIV+ individuals[134, 140].

The above indicated discrepancy could mirror the diverse effects of HIV infection on immune responses. It is also possible that it is linked to the fact that all studies have measured cure rates and rates of reinfection using egg microscopy which may lack sensitivity, is not a direct measure for worm burden, and might be affected by HIV infection.

B. Effect of HIV on egg excretion

Regarding the impact of HIV on *Schistosoma* spp. infection, there is no consensus as to whether *Schistosoma* spp. egg excretion is lowered in those with HIV infection as compared to those without[76, 78, 88, 99, 101, 126, 127]. Studies that looked at the circulating worm antigens in relation to HIV status indicated no relevant differences between the groups[88, 101, 110, 126].

In **Chapter 6**, we investigate the reasons for the discrepancies found regarding the impact of HIV infection on *Schistosoma* spp. excretion of eggs. Based on the difference in clinical disease in men and women, we hypothesized that sex would be a main confounder in the relationship between HIV status and *Schistosoma* spp. egg excretion.

Table 2 - Relationship between HIV infection and *Schistosoma* egg excretion and treatment.

Reference	Total sample size (% schisto)	Study type	Schisto species	Schisto test	Population	ART	Findings
(1) Egg excretion							
76. Mazigo HD et al., 2014. Parasit Vectors	1785 (47.8%)	Cross-sectional	*Sm*	Kato-Katz	Humans Adults Male and female	NA	No difference in egg excretion
99. Kleppa E et al., 2015. PLoS One	765 (20%)	Cross-sectional	*Sh*	Urine microscropy	Humans High-school students >16 yo Female only	Some on ART – not adjusted for	No difference in egg excretion
125. Olusegun AF et al., 2011. Oman Med J	2000 (0.3%)	Cross-sectional	*Sh*	Urine microscopy	Humans Adults Male and Female	NA	No difference in egg excretion
78. Sanya RE et al., 2015. Trop Med Int Health	1412 (57.2%)	Cross-sectional	*Sm*	Kato-Katz	Humans All ages >13 yo Male and Female	NA	Reduced egg excretion
101. Kallestrup P et al., 2005. J Infect Dis	1545 (43.4%)	Cross-sectional	All	Microscopy CAA	Humans Adults Male and Female	ART not widely available at time of study	Reduced egg excretion
126. Fontanet AL et al., 2000. Ann Trop Med Parasitol	1239 (31.4%)	Cross-sectional	*Sm*	Kato-Katz CAA	Humans 15-54 yo Male and Female	NA	Reduced egg excretion
88. Karanja DM et al., 1997. Am J Trop Med Hyg	53 (100%)	Cross-sectional	*Sm*	Microscopy CCA	Humans Adults Male only	NA	Reduced egg excretion

127. Mwanakasale V et al., 2003. Am J Trop Med Hyg	507 (100%)	Cross-sectional	*Sh*	Urine microscopy	Humans 10-55 yo Male and Female	NA	Reduced egg excretion
(2) Praziquantel efficacy							
127. Mwanakasale V et al., 2003. Am J Trop Med Hyg	507 (100%) at baseline	-Longitudinal study -Treatment with PZQ -3 follow-ups up to 12 months	*Sh*	Urine microscopy	Humans 10-55 yo Male and Female	NA	No difference in PZQ efficacy
138. Mazigo HD et al., 2014. Infect Dis Poverty	555 (100%)	-Longitudinal study -Treatment with PZQ -Only one follow-up 12 weeks post-treatment	*Sm*	Kato-Katz	Humans Adults Male and Female	ART Naive	No difference in PZQ efficacy
139. Karanja DM et al., 1998. Am J Trop Med Hyg 59: 307-11.	47 (100%)	-Longitudinal study -Treatment with PZQ -Two follow-ups, up to 6 months	*Sm*	Microscopy CCA	Humans Adults Male only	NA	No difference in PZQ efficacy
140. Kallestrup P et al., 2006. Clin Infect Dis	287 (100%)	-Longitudinal -Treatment with PZQ -3 follow ups up to 12 months	All	Microscopy	Humans Adults Male and Female	ART not widely available	Lower PZQ efficacy
134. Karanja DM et al., 2002. Lancet	107 (100%)	-Longitudinal -Treatment with PZQ -Follow-ups every 2 months for at least 1 year	*Sm*	Microscopy	Humans Adults >17yo Male only	NA	Lower PZQ efficacy

Schisto = *Schistosoma*
Sm = *S. mansoni*
Sh = *S. haematobium*
NA = Not available or not applicable

SUMMARY

- 6 million individuals are HIV/*Schistosoma* spp. co-infected
- There is a clear association between *Schistosoma* spp. and HIV
- Being *Schistosoma* spp. infected increases a woman's risk of HIV acquisition
- A lot is still unknown or poorly understood about HIV/*Schistosoma* spp. co-infections
- Longitudinal studies, controlling for sex, age, and duration of HIV infection are missing

Questions that this thesis is trying to answer:

✓ Why is there so much discrepancy in the data?

✓ Is there an impact of *Schistosoma* spp. co-infection on HIV/AIDS outcomes?

✓ Is there an impact of *Schistosoma* spp. co-infection on HIV transmission?

- Thesis studies were conducted in the Lake Zone of Tanzania

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CHAPTER 2 - CASCADE OF CARE FOR HIV-SEROCONVERTERS IN RURAL TANZANIA: A LONGITUDINAL STUDY.

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ABSTRACT

We examined the HIV care cascade in a community-based cohort study in Kisesa, Magu, Tanzania. We analyzed the proportion achieving each stage of the cascade - Seroconversion, Awareness of HIV status, Enrollment in Care and Antiretroviral therapy (ART) initiation- and estimated the median and interquartile range for the time for progression to the next stage. Modified Poisson regression was used to estimate prevalence risk ratios for enrollment in care and initiation of ART.

From 2006 to 2017, 175 HIV-seroconverters were identified. 140(80%) knew their HIV status, of whom 97(69.3%) were enrolled in HIV care, and 87(49.7%) had initiated ART. Time from seroconversion to awareness of HIV status was 731.3[475.5-1345.8] days. Time from awareness to enrollment was 7[0-64] days, and from enrollment to ART initiation was 19[3-248] days. There were no demographic differences in enrollment in care or ART initiation. Interventions that increase enrollment are likely to have the most impact in achieving the Joint United Nations Programme on HIV and AIDS targets.

Keywords: HIV Care Continuum, Linkage to care, Testing, ARV, Tanzania

INTRODUCTION

Widespread availability of antiretroviral therapy (ART) has led to tremendous declines in HIV/AIDS related mortality, especially in Africa. By 2015, 10.3 million people (54% of those with HIV infection) were accessing ART in sub-Saharan Africa[1], with a reported 50% decrease in crude death rates among people living with HIV (PLHIV) following the introduction of ART in eastern and southern Africa[2]. In North-west Tanzania, mortality in HIV-infected people declined by a third between the mid 1990s and 2004[3].

Despite these important declines, mortality among HIV-infected adults remains unacceptably high, with a crude death rate among HIV-infected adults three times higher than non-infected adults in 2009–11 in eastern and southern Africa[2]. Most of deaths in HIV-infected individuals are still due to tuberculosis and HIV/AIDS, suggesting sub-optimal use of HIV services[2]. In Northwest Tanzania, although overall incidence and prevalence had reduced slightly, both are consistently higher in the age group 35-44 years, likely due to poor linkage to care allowing on-going transmission (unpublished results).

This indicates substantial room for improvement in current services in order to promote the benefits of earlier HIV testing and to encourage access to care for those diagnosed positive. Even among HIV-infected individuals who know their status, a large proportion do not enroll into care and treatment[1-3].

The target for ART programs worldwide has been defined by the Joint United Nations Programme on HIV and AIDS (UNAIDS) using the slogan 90-90-90 targets[4]. This aims to achieve 90% of PLHIV diagnosed (knowing their status), 90% of those diagnosed initiated on ART, and 90% of individuals on ART being virologically suppressed. Reaching the UNAIDS targets requires early diagnosis and effective linkage to and retention in care[5, 6]. Not only is it important to be aware of the presence and nature of obstacles to care, but identification of key factors associated with linkage to care are also needed to improve benefits of ART through the continuum of care[7].

We used data from a community-based cohort in Tanzania to assess the spectrum of engagement in care of PLHIV in Northwest Tanzania and estimate the achievement of the 90-90-90 targets in this population.

METHODS

Study population

The data for this paper is derived from a community-based cohort study in Kisesa, Tanzania, covering an area of about 115 km2 with 35000 inhabitants residing in the study area[3,8,9]. Approximately every 3 years all residents aged 15 years or more are invited to a sero-survey to determine the health needs in the population, with the first in 1994, and the 8th sero-survey finished in February 2016. All sero-survey participants were offered Voluntary Counseling and Testing (VCT), and if found to be HIV-infected were referred to the main clinic in Kisesa for treatment. Dried blood spot samples (DBS) from all consenting participants, whether or not they had VCT, were tested for HIV-1 at the National Institute for Medical Research (NIMR) reference laboratory in Mwanza. We identified seroconverters from HIV testing of DBS between September 2006 (sero-survey 5) and February 2016 (sero-survey 8). Seroconverters were defined as those with at least one HIV negative test, and a subsequent HIV positive test, with the date of seroconversion defined as the mid-point between dates of the last negative test and the first positive test.

Three stages of the HIV care cascade were included in our framework:

1) Awareness of HIV status: defined as the awareness of positive HIV status either through VCT at the sero-survey, or through self-reported HIV Counseling and Testing (HCT) attendance or other proof of HCT (available in public health facilities unrelated to the sero-surveys). The date of awareness of HIV status was defined as the date the person first knew of his or her own HIV status.

2) Enrollment in HIV care: defined as completing and/or self-reporting at least 1 visit to a Care and Treatment Clinic (CTC). Those diagnosed with HIV through a positive HIV test at any of the HCT clinics, or through VCT in the sero-surveys, are referred to a CTC. The date of CTC enrolment was defined as the date of the first reported attendance at CTC.

3) ART initiation: defined as having a clinically confirmed ART initiation report and/or self-reported use of ART. Until 2010, the criteria for ART initiation were a CD4 count ≤200 cells/mm3 or a WHO clinical stage of 4 for all adults. The criteria changed to ≤350 cells/mm3 from 2010-2012, ≤500 from 2013-2015, and all HIV-infected in 2016[10-13]. The date of ART initiation was defined as the first reported date that ART was given to participants.

Follow-up

The follow-up period spanned from date of seroconversion to March 15th 2017, the date at which everyone who had not progressed to the next stage was censored. We searched for each seroconverter manually and via a record linkage computer algorithm using name, sex, date of birth, and place of residence in all the health clinics providing HIV care within a 10

km radius around the sero-survey catchment area. We additionally visited the two oldest and largest HIV clinics in the region (in Mwanza City, 20 km from the DSS) to search for seroconverters.

Statistical analysis

The proportion of HIV-positive persons achieving each stage in the cascade was calculated. Statistical inference for differences between levels of explanatory variables was based on a $\chi 2$ test for categorical variables. We also used modified Poisson regression to estimate prevalence risk ratios (PRRs) and 95% confidence intervals (95%CIs) for enrollment in care and initiation of ART. All plausible variables were individually included into the model and model goodness-of-fit assessed. Data were entered into Microsoft Excel and all analyses were performed in STATA 14.1 (College Station, TX, USA).

Ethical considerations

Ethical approval for retrospective analysis of these data was obtained from Bugando Medical Centre in Mwanza (BREC/001/04/2011), the National Institute for Medical Research in Dar es Salaam (NIMR/HQ/R.8a/Vol.IX/1489), and Weill Cornell Medicine in New York (1108011883). Study participants provided consent at the time of enrollment into the cohort study in accordance with the approved procedures of the TAZAMA project[8, 9].

RESULTS

From September 2006 to February 2017, a total of 207 HIV-seroconverters were identified in the cohort. Among those, 175 HIV-seroconverters were available for follow-up. A total of 20 health facilities (HCTs and CTCs) contributed to the analysis. All facilities are government-run. All provide HIV testing and 10/20 (50%) provide CTC services, including monthly disease monitoring, provision of ART and TB screenings.

The demographics of the HIV-seroconverters are presented by stage of the cascade in **Table 1**. There was no difference in the proportions of HIV-seroconverters by area of residence.

As of March 2017, end of follow-up, 140/175 (80.0%) knew their HIV status, 97/175 (55.4%) were enrolled in HIV care, and 87/140 (62.1%) had initiated ART. The cascade of care is presented in **Figure 1**.

Table 1 - Proportion of the 175 HIV-seroconverters engaged in each of the HIV care cascade stages by selected characteristics.

Characteristic	HIV seroconverters	Aware of HIV status		Enrolled in care		Initiated ART	
	n (%)	n (%)	p-value[a]	n (%)	p- value[a]	n (%)	p-value[a]
Total	175	140 (80.0%)	-	97 (55.4%)	-	87(49.7%)	-
Sex							
Female	118 (67.4%)	93 (78.8%)	0.57	69 (58.5%)	0.24	63 (53.4%)	0.16
Male	57 (32.6%)	47 (82.5%)		28 (49.1%)		24 (42.1%)	
Occupation							
Farmer	142 (81.1%)	111 (78.2%)	0.42	74 (52.1%)	0.17	69/142 (48.6%)	0.77
Businessman	12 (6.9%)	11 (91.7%)		9 (75.0%)		6/12 (50.0%)	
Mix of farming and business	21 (12.0%)	18 (85.7%)		14 (66.7%)		12/21 (57.1%)	
		Median (IQR)	**p-value[a]**	**Median (IQR)**	**p-value[a]**	**Median (IQR)**	**p-value[a]**
Age							
Aware/enrolled/initiated	36 (27-46)	36.5 (28-46)	0.46	35(27-43)	0.15	36.5 (27.5-48)	0.42
Not aware/enrolled/initiated		34 (26-43)		37(28-52)		36 (27-45)	
Years of education							
Aware/enrolled/initiated	7(2-7)	7(2-7)	0.75	7(0-7)	0.16	7 (3.5-7)	0.062
Not aware/enrolled/initiated		7(0-7)		7(3-7)		7 (0-7)	

[a]*Use of Chi-square test for categorical variables and rank-sum test for continuous variables.*

Figure 1 - Cascade of care in the study population, Tanzania. The first two 90-90-90 targets are in bold.

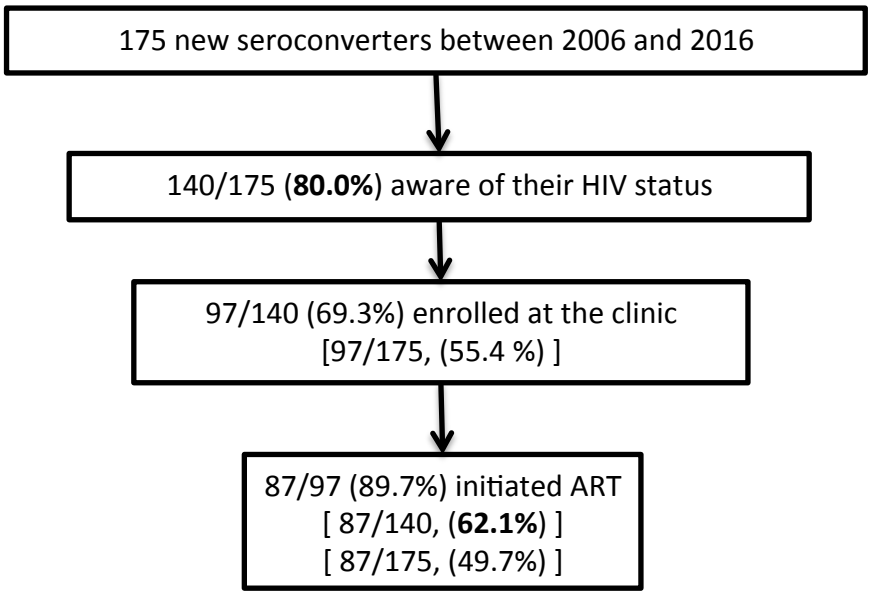

Figure 1 depicts the cascade of care in our study population. 80% of HIV-infected people knew their HIV status and 62% of these were on ART.

The majority of the seroconverters learned about their positive status for the first time at the sero-survey (67/140, 47.8%), or at a HCT clinic (47/140, 33.6%). 20/140 (14.3%) of the seroconverters discovered their status via provider-initiated testing and 6/140 (4.3%) at Ante-Natal Care (ANC).

Out of the 97 individuals who had reached a CTC, 19 of them migrated or had transferred. 12/97 (12.4%) transferred from one clinic to another within the 20 clinics searched, mostly after 2008 when new CTCs opened. 1 out of 97 deliberately opted out of CTC, 4 out the 97 were reported as transferred out of the clinic and moved outside the catchment area and 2 temporarily transferred care to a larger CTC clinic during their pregnancies.

The median time from seroconversion to being aware of one's HIV status was 731.3[475.5-1345.8] days while time from awareness of HIV status to enrollment was 7[0-64] days. The overall median time from enrollment to ART initiation was 19[3-248] days. The key time variables are presented in **Table 2**.

Table 2 - Key time variables (in days) for the 175 seroconverters.

Variable	Median	IQR	Min	Max	N
Time from seroconversion to awareness	731.3	475.5 - 1345.8	135.5	2889	140
Time from seroconversion to enrollment	965.5	511.5 - 1652.5	135.5	2889	93[a]
Time from seroconversion to ART initiation	1247.5	803.5 - 1867.5	206.5	3324.5	83[a]
Time from awareness to enrollment	7.0	0 - 64	0	2170	93[a]
Time from awareness to ART initiation	146.0	19 - 535	0	2989	83[a]
Time from enrollment to ART initiation	19.0	3 - 248	0	2988	83[a]
Time being followed-up at the clinic (up to March 15th 2017)	803.0	378 - 1646	64	3009	93[a]

IQR= Interquartile range; Min=minimum; Max=maximum

[a] *4 people self-reported attending a clinic and initiation ART but did not provide us with exact dates.*

Time to ART initiation decreased sharply with the successive implementation of new guidelines. Time from awareness of HIV status to enrollment transiently increased with the creation of new clinics where old seroconverters who had not previously been enrolled in care could now enroll in care more easily. Throughout the study period, the time from awareness of HIV status to enrollment was longer for those receiving their results at sero-surveys than for those receiving their results at a clinic ($p<0.001$). These results are presented in **Figure 2**.

Figure 2 - Variations in reaching each stage of the cascade by year of enrollment.
A) Timeline of the different guidelines and sero-surveys
B) Boxplot of time from seroconversion to awareness of HIV status by year of enrollment
C) Boxplot of time from awareness of HIV status to enrollment in a CTC by year of enrollment
D) Boxplot of time from enrollment in a CTC to initiation of ART by year of enrollment

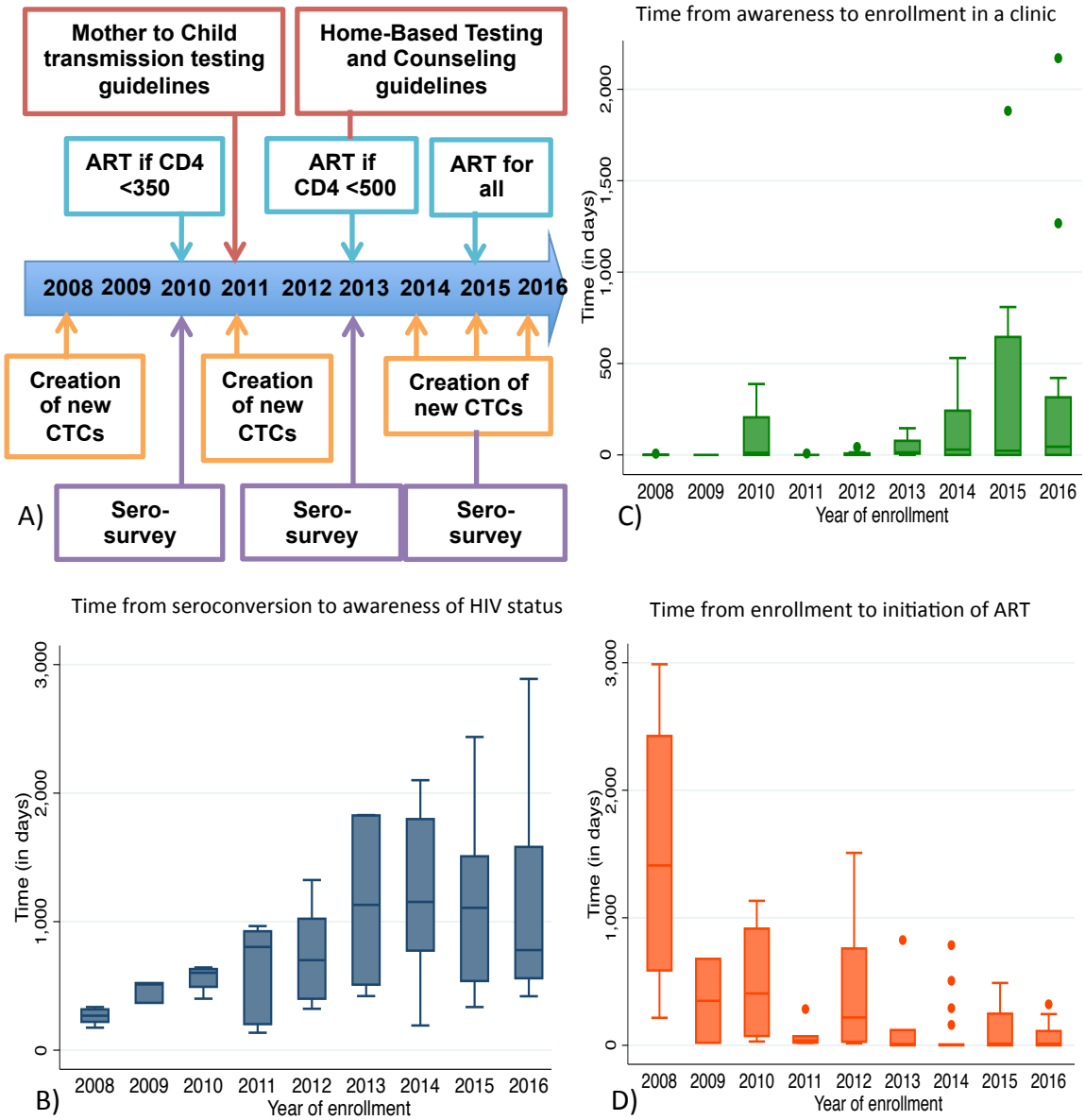

Figure 2 shows the variation in key time variables over the years, following major HIV/AIDS testing and care interventions. Time to ART initiation decreased dramatically with the successive implementation of new guidelines while time from diagnosis to enrollment transiently increased with the creation of new clinics. The first sero-survey was conducted in 2006-2007 and is not represented on this figure.

There was no difference by sex in enrollment in care (adjPRR=0.85, 95%CI 0.61-1.17), and no other significant differences. There was also no difference by sex in ART initiation (adjPRR=0.75, 95%CI 0.51-1.08), and no other significant differences.

DISCUSSION

This is the first report, to our knowledge, on Tanzania's achievements regarding the 90-90-90 targets. This longitudinal study in Tanzania occurred at a unique time during which new HIV clinics were opening and ART eligibility was increasing. Our Tanzanian study population demonstrates some moderate successes, with 80% of HIV-infected people knowing their HIV status and 62% of these on ART. Routine viral load monitoring was not yet fully implemented at the time of our study and future studies will evaluate the 3rd target of the UNAIDS 90-90-90 goal.

The success in our rural study population is that the percentage of HIV-positive individuals knowing their status is high. This effect was largely driven by the recent strategy change of the final sero-survey in 2016, which provided opt-out HIV testing for all participants rather than requiring the person providing blood to take initiative to obtain their results. This highlights the need for more regular access to opt-out testing and a greater encouragement for uptake of HIV testing[1]. Due to the logistical challenges and the expense of large-scale sero-surveys, provider-initiated testing might be more able to fit those criteria[14].

Very few women in our study population received their HIV results through antenatal testing. No other demographic factor predicted enrollment into care or initiation of ART, which strongly suggests that failure in linkage to care is inherent to the system, and not due to patient factors[1,15]. There is also a possibility that our team was not able to find everyone in the clinics, either because they moved out of the region, or because they changed names at the clinic to avoid stigma.

Still too few people are initiating ART. The low percentage of diagnosed individuals beginning care and treatment can be explained partially by the fact that not many easily accessible CTCs were opened until 2014. Thus the increasing time from seroconversion to enrollment in care over the years is indeed the result of more old seroconverters enrolling in CTCs as they open. This suggests that as access to care is expanded, linkage to care in our population will continue to increase. Importantly, more CTCs continue to open in the region and the country and home-based testing is also currently being investigated in Tanzania[16]. Both of these will likely serve as powerful resources to strengthen linkage of HIV-infected individuals to testing and care.

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CHAPTER 3 – IMPACT OF SCHISTOSOME INFECTION ON LONG-TERM HIV/AIDS OUTCOMES

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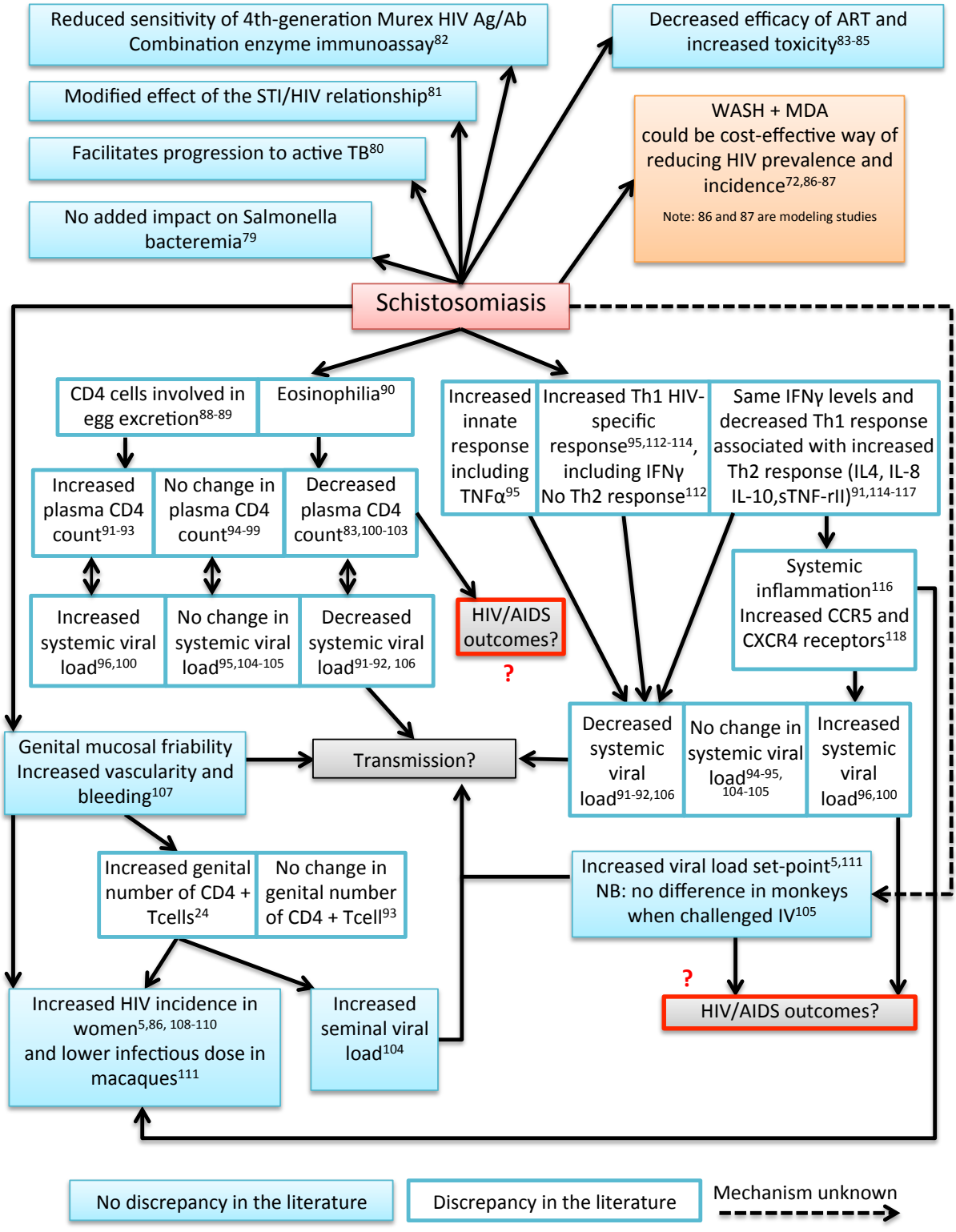
WHERE DOES THIS CHAPTER FIT?



ABSTRACT

Africa bears the burden of approximately 70% of global HIV infections and 90% of global schistosome infections. We sought to investigate the impact of schistosome infection at the time of HIV-1 seroconversion on the speed of HIV-1 disease progression, as measured by the outcome CD4+ T-cell (CD4) counts <350 cells/μL and/or death. We hypothesized that people who had been infected with *Schistosoma* spp. at the time they acquired HIV-1 infection would have impaired antiviral immune response, thus leading them to progress twice as fast to a CD4 count less than 350 cells/μL or death than would people who had been free of schistosomes at time of HIV-1 seroconversion. We conducted a longitudinal study in Tanzania from 2006 to 2017 using stored blood spot samples, demographic surveillance and sero-survey data from the community, and a review of clinical charts. A competing risk analysis was performed to look at the difference in time to reaching CD4 counts < 350 cells/μL and/or death in HIV-1-infected people who were infected versus not infected with *Schistosoma* spp. at time of HIV-1 seroconversion. We found an 82% reduction in risk of reaching the outcome in seroconverters who had been infected with *Schistosoma* (subHazard Ratio = 0.18[0.068,0.50], p = 0.001) after adjusting for age, occupation, clinic attendance and time-dependent covariates. Our study demonstrates that people with schistosome infection at the time of HIV-seroconversion develop adverse HIV outcomes more slowly than those without. The findings are contrary to our original hypothesis. Our current longitudinal findings suggest complex interactions between HIV-1 and schistosome co-infections that may be modulated over time. We urge new immunological studies to investigate the long-term impact of schistosome infection on HIV-1 viral load and CD4 counts as well as related immunologic pathways.

Keywords: *Schistosoma* spp, HIV, AIDS, co-infection, ART, competing risk analysis

INTRODUCTION

Among approximately 36.7 million global HIV infections, an estimated 6 million individuals are schistosome co-infected[1–3]. Multiple studies have reported interactions between infection with *Schistosoma* spp. and HIV-1 in humans. A recent longitudinal study in Tanzania demonstrated that schistosome infection is a risk factor for HIV-1 acquisition in women, but not in men[3]. These prospective findings substantiated four different cross-sectional studies from Tanzania and Zimbabwe that had shown a ~3-fold increased odds of HIV-1 infection in women with schistosome infections compared to those without, and no increased odds in men[4–7]. Local physical and immunological changes caused by schistosome eggs in the mucosal tissue of the vagina and cervix are thought to increase susceptibility to the virus during sexual HIV exposure[8–10], providing a mechanism for how schistosome infection could increase the risk of incident HIV-1 infection in women. In contrast, men with schistosome infections may not have increased susceptibility to HIV-1 infection because schistosome eggs in men primarily affect internal genital organs, such as the prostate, that are not exposed to HIV-1 during sexual contact[7, 11, 12].

In addition to demonstrating increased HIV-1 susceptibility, the longitudinal study in Tanzania also found increased HIV-1 RNA viral load set-points in both men and women who were infected with *Schistosoma* spp. at time of HIV-1 seroconversion[3]. Some, though not all, additional studies have demonstrated that treatment of *Schistosoma mansoni* is associated with a decrease in HIV-1 viral load in co-infected individuals[8, 13]. Therefore, it is conceivable that schistosome infection at the time of HIV-1 acquisition increases the HIV-1 viral load set-point, which in turn could accelerate AIDS-related-outcomes[14]. The median increase of 0.7 $\log_{10}$ copies/mL that was observed in the study would be predicted to increase the time to AIDS or death by 2 to 3 years[3, 15].

We thus sought to investigate the impact of schistosome infection at the time of HIV-1 seroconversion on the speed of HIV-1 disease progression, as measured by CD4+ T-cell (CD4) counts and mortality. We hypothesized that people who had been infected with schistosomes at the time they acquired HIV-1 infection would have impaired antiviral immune response, thus leading them to progress twice as fast to a CD4 count less than 350 cells/μL or death compared to people who had been free of schistosomes at time of HIV-1 seroconversion.

METHODS

Study setting and design

Identification of HIV-seroconverters - Our study was conducted within the ongoing TAZAMA project, a community-based longitudinal open HIV-testing cohort in Kisesa, northwest Tanzania, which has conducted sero-surveys in a population of ~20,000 adults since 1994. Adults are offered voluntary HIV testing and counseling and provide dried blood spots (DBS) every three years. A Demographic Surveillance System documents population characteristics, births, migration, and deaths every six months. Additional details have been previously described[16, 17]. For this study, we identified seroconverters who became HIV-1-seropositive between September 2006 (sero-survey 5) and February 2016 (sero-survey 8), our enrollment period, and who had archived DBS or serum available for testing. Seroconverters were defined as individuals who had been HIV-1-seronegative in one sero-survey and who were found to be HIV-1-seropositive in a following sero-survey. Seroconverters' demographic data was obtained through linkage to the demographic surveillance data.

Follow-up - The follow-up period spanned from date of seroconversion to March 15th 2017. The date of seroconversion was approximated as the mid-point between the last negative DBS and the first positive test, either at a sero-survey or at a clinic. In order to assess the clinical outcomes of HIV-1 seroconverters, we searched for each seroconverter manually and via computer algorithm, by name, sex, date of birth, and place of residence in all the health clinics providing HIV care within a 10 km radius around the sero-survey catchment area. We additionally visited the two oldest and largest HIV clinics in the region (in Mwanza City, 20 km from the demographic surveillance system area) to search for seroconverters. The demographic surveillance data were also used to obtain vital status of HIV-1-seroconverters.

Data about patients seeking care for HIV infection was extracted from both paper files and computer databases. Data that was collected included antiretroviral treatment use and adherence, co-infection with tuberculosis, WHO-defined HIV/AIDS clinical stage, CD4 count, weight, co-infection with other sexually transmitted infections, and comorbidities such as self-reported hypertension and diabetes.

Since 2004, CD4 count monitoring has been used to assess ART eligibility. Until 2010, the criteria for ART initiation were a CD4 count ≤200 cells/mm3 or a WHO clinical stage of 4 for all adults. From 2010 to 2012, the criterion was CD4 count ≤350 cells/mm3. From 2013 to 2015, it changed to CD4 count ≤500 cells/mm3 and finally in 2016 any HIV-positive individual was eligible to initiate ART[18–21].

Schistosome infection status - Determination of schistosome infection status was made by measurement of schistosome Circulating Anodic Antigen (CAA) in DBS collected during two successive sero-discordant sero-survey visits. The CAA test is a genus specific assay and thus does not differentiate between *mansoni* and *haematobium* species present in the Kisesa

area of Tanzania. Our group has previously shown that approximately 40% of the adults have *S. mansoni* infection and 2% have *S. haematobium* infection[5, 7].

We defined schistosome positivity at time that a person became HIV-1 infected as having a positive test for schistosome infection in that person's DBS collected both at the last sero-survey where he/she tested negative for HIV-1 and at the first sero-survey where he/she tested positive for HIV-1. If at least one of the two DBS was negative for schistosome infection, the individual was defined as schistosome negative at time of HIV-1 sero-conversion.

Laboratory Testing

Dried blood spots - DBS were collected by finger prick with a fingerstick lancet onto a Whatman Protein Saver 903 card (GE Healthcare Bio-Sciences, Pittsburgh, PA). Each spot of blood is 13 millimeters in diameter. DBS cards were dried out of direct sunlight and sealed in a gas-impermeable zip bag with desiccant and humidity indicator. Cards were stored at the NIMR laboratory in Mwanza at -20°C.

HIV-1 testing - Diagnosis of HIV-1 infection was confirmed by two different tests in accordance with current national HIV guidelines at each time point. In sero-surveys 5 and 6, the Uniform II Category III Ab test was used as the screening test and the Enzygnost test was used as the confirmatory test. In sero-survey 7, the Uniform II Category IV Ab+Ag test was used as the screening test and Enzygnost was used as confirmatory test. In sero-survey 8, the Determine test was used as the screening test and the Unigold test was used as the confirmatory test. For sero-surveys 5 to 7, DBS were tested at the NIMR laboratory. For sero-survey 8, rapid tests were used on site for screening and confirmation of HIV-1, and around 10% of the stored DBS were tested at NIMR as quality check for the rapid test results. If the sample was negative at the screening test the final result was reported as negative. A sample that was positive at the screening test was tested with the confirmatory test. If the confirmatory test was negative, the final result was reported as negative. If the confirmatory test was positive, the final result was reported as positive. At the Bugando Medical Centre clinical laboratory in Mwanza, Tanzania, CD4 counts were measured using an automated BD FACS Calibur Machine (BD Biosciences, San Jose, CA, USA).

Schistosoma *spp. testing* - DBS were tested for schistosome CAA at Leiden University Medical Center by eluting whole blood from DBS and then concentrating the sample as previously described[22], with minor modifications. A total of 226 mm2 of DBS were placed into 500µL of phosphate-buffered saline and incubated for 30 minutes at room temperature and then overnight at 4°C. The next day, samples were placed on a shaker for 30 min at room temperature and 30 min at 37°C, after which 250µL of 6% (w/v) trichloroacetic acid (TCA) was added. The mixture was vortexed, centrifuged, and concentrated using an Amicon 0.5 mL concentration device (Merck, Darmstadt, Germany). The concentrate was then used in the standard CAA UCP assay.

A lower limit threshold of 2 pg CAA per mL of eluted blood was used for the assay. Eleven individuals had stored serum samples but no DBS samples available for testing and underwent serum CAA testing at NIMR with a lower limit threshold of 30 pg CAA per mL[23]. Samples scoring values above the threshold were designated positive for *Schistosoma* infection.

Statistical analysis

Analysis included all individuals who HIV-1 seroconverted between September 2006 and February 2016. Binary variables were described as proportions and continuous variables were described using median and interquartile range. We assessed differences in baseline clinical characteristics between schistosome positive seroconverters and schistosome negative seroconverters using Chi-square or Fisher's exact test for proportions and the nonparametric equality test for medians.

A competing risk analysis was conducted to look at the difference in time to outcome in HIV-1 seroconverters who were infected versus not infected with *Schistosoma* at time of HIV-1 seroconversion. The outcome of interest was defined as a composite endpoint: either CD4 count <350cells/μL or death. The competing risk event was defined as start of antiretroviral treatment (ART) when occurring without a preceding outcome, since after ART initiation the risk of reaching the outcome of interest becomes very small. Data was censored for loss to follow-up, defined as the latest of 3 months after the last clinic visit or 1 year after the last demographic surveillance visit. All study participants were censored on 15th March 2017 for this analysis. The cumulative incidence function method was used to assess and compare time to outcome between schistosome infection groups. A competing risk regression with subdistribution hazard analysis, adjusted for all significantly different baseline factors as well as biologically sound variables, was used to assess endpoint incidence difference by schistosome infection status while controlling for ART initiation. Variables that were associated with the outcome at a 10% significance level were individually included into a step-wise analysis and model goodness-of-fit assessed.

Using the methods of Latouche et al.[24] to calculate sample size for subdistribution hazard ratio with competing risk, we predicted that we needed 325 subjects to obtain 91 occurrences of the outcome (CD4 count<350 cells/μl or death) for calculating a sub-hazard ratio of 2 (SHR = 2.0), as significant, with 95% confidence intervals.

Validity of the proportional hazards assumption was tested by including time-dependent covariates in the model, namely the last time seen at a demographic surveillance visit and the last time seen in a clinic. To account for the effect of missing follow-up clinical data (such as ART initiation and CD4 counts), we assessed differences in outcome in those found in clinics compared to those not found in clinics. We pre-specified that we would keep a variable for not being found in a clinic in all models if significant. Finally, a sensitivity analysis was done to assess for bias due to loss-to-follow-up by considering all lost-to-follow-ups as reaching the composite endpoint. Another sensitivity analysis was done to assess for bias due to our

definition of *Schistosoma* spp. infection at time of HIV-1 seroconversion. For this sensitivity analysis, we assessed the effect of defining *Schistosoma* spp. infection as having the pre-seroconversion DBS positive for *Schistosoma* spp., instead of requiring both DBS to be positive.

Data were entered into Microsoft Excel and all analyses were performed in STATA 14.1 (College Station, TX, USA). When exact dates were not available, dates were approximated to the 15th of the month if only the month was known or to the 1st of July if only the year was known. All results were expressed with 95% confidence intervals (CIs) and statistical significance was set at $P < 0.05$ (two-tailed).

Ethical considerations

Ethical approval for retrospective and prospective analysis of these data was obtained from Bugando Medical Centre in Mwanza (BREC/001/04/2011), the National Institute for Medical Research in Dar es Salaam (NIMR/HQ/R.8a/Vol.IX/2446), and Weill Cornell Medicine in New York (1108011883). Study participants provided consent at the time of enrollment into the cohort study in accordance with the approved procedures of the TAZAMA project, which included consent for future testing of dried blood spot samples[16, 17].

RESULTS

Between September 2006 and February 2016, 172 adults aged 18 years and above HIV-1-seroconverted within the TAZAMA sero-surveys and had stored pre- and post-seroconversion samples available for testing for *Schistosoma* infection. A total of 63/172 (36.6%) seroconverters had a pre-HIV-seroconversion positive test for *Schistosoma*. 43/172 (25.0%) had both samples positive for *Schistosoma*. These 172 HIV-1 seroconverters were followed for a median of 3.4 [2.3–5.4] years from the time of seroconversion to censoring or completion of the study. 98 (57.0%) were found in 16 of the 20 HIV clinics visited and the remaining 74 were found in the demographic surveillance data. 82(85.4%) were known to have initiated ART by March 2017. The baseline characteristics of all seroconverters are presented in **Table 1**.

Table 1 - Demographics of the TAZAMA HIV-1 seroconverters, by schistosome infection status at time of HIV-1 seroconversion.

Variable		Schistosome infected N=43	Schistosome uninfected N=129	p-value
Female		26/43 (60.5%)	90/129 (69.8%)	0.26
Age in years at HIV-1 seroconversion (Median-IQR)		34[27-47]	35[26-43]	0.79
Smoking		5/36 (13.9%)	12/109 (11.0%)	0.64
	Never	30/36 (83.3%)	96/109 (88.1%)	0.48
	Less than once a month	2/36 (5.6%)	4/109 (3.7%)	
Alcohol	1-3 days per month	1/36 (2.9%)	5/109 (4.6%)	
consumption	1-4 days per week	3/36 (8.3%)	2/109 (1.8%)	
	5-6 days per week	0/36 (0.0%)	1/109 (0.9%)	
	Every day	0/36 (0.0%)	1/109 (0.9%)	
Reported sexually transmitted infection		23/40 (57.5%)	75/126 (59.5%)	0.82
Reported hypertension		1/31 (3.2%)	4/84 (4.8%)	0.72
Tuberculosis positive		2/21 (9.5%)	3/73 (4.1%)	0.33
More than 7 years of education		27/43 (62.8%)	80/129 (62.0%)	0.93
Found in an HIV clinic		23/43 (53.5%)	75/129 (58.1%)	0.59
CD4 < 350 cells/uL		6/23 (26.1%)	33/75 (44.0%)	0.12
Reported death (from demographic surveillance system or clinic)		2/43 (4.7%)	9/129 (7.0%)	0.59
Initiated antiretroviral treatment		21/22 (95.5%)	61/73 (83.6%)	0.13

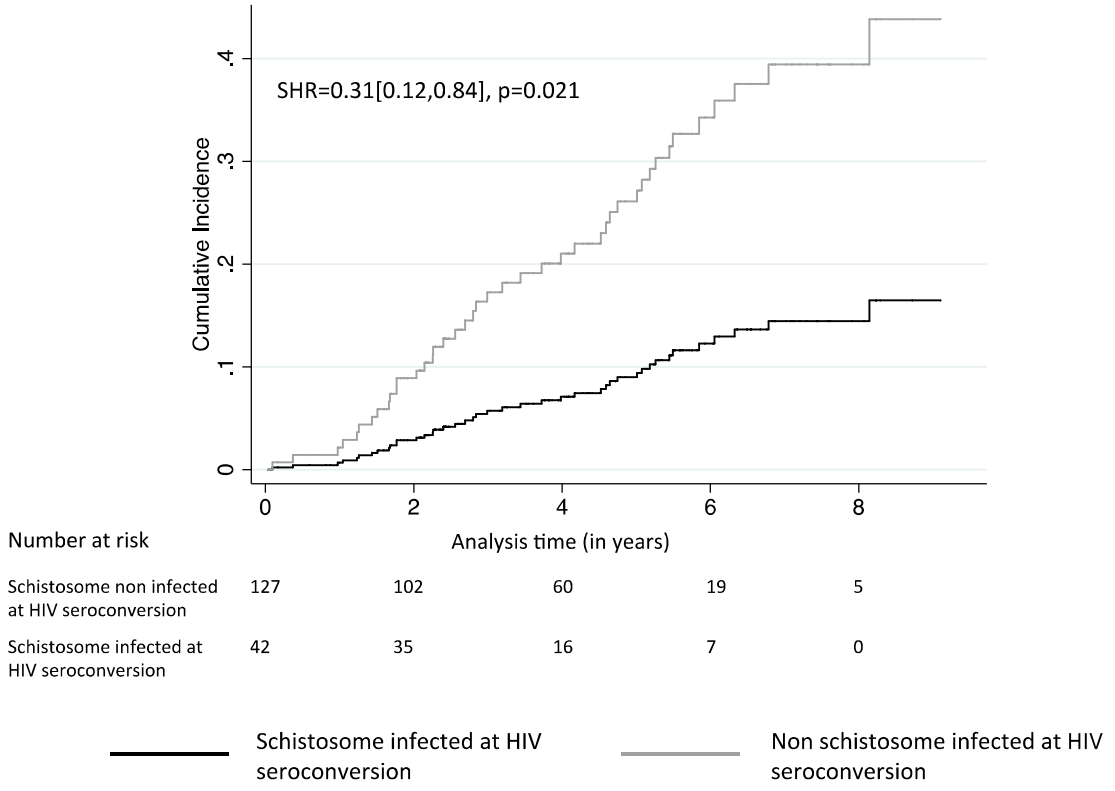
There were 42 occurrences of the outcome (defined as either reaching CD4 count <350cells/μL or death) in 636.4 person-years of follow-up (4 occurrences in 142.2 person-years for schistosome positive seroconverters and 38 occurrences in 494.2 person-years for schistosome negative seroconverters). 50 adults had initiated ART before reaching a CD4 count <350 cells/μL and therefore had experienced the "competing risk" (defined as starting antiretroviral treatment before reaching the outcome). 35 of the 39 CD4 counts <350 cells/μl occurred before the person had initiated ART and 7 of the 9 deaths happened in adults that had not reached a CD4 count<350 cells/μL and had not started ART, yielding the total of 42 occurrences of the outcome that occurred prior to ART initiation. Schistosome positive seroconverters had experienced the competing risk 33 times while schistosome negative seroconverters had experienced the competing risk 17 times. The differences in the outcome and competing risks are presented in **Table 2**.

Table 2 - Results of the univariable competing risk regression based on sub-distribution hazard ratios.

Variable		Person-time (in years)	Number of occurrences of the outcome	Number of competing events	Sub-Hazard Ratio [95%CI]	p-value
***Schistosoma* spp.**	Negative	494.2	38	33		
(ref= CAA negative at HIV-1 seroconversion)	Positive	142.2	4	17	0.31 [0.12,0.84]	0.021
Before/After last seen at a demographic surveillance visit	Before	602.4	31	32		
(ref=before)	After	34.0	11	18	4.14 [1.86,9.24]	0.001
Before/After last seen at a clinic	Before	328.2	35	50		
(ref=before)	After	308.2	7	0	0.31 [0.14,0.71]	0.005
Attending clinic	No	282.9	5	0		
(being located in a regional clinic)	Yes	353.5	37	50	4.09 [1.60,10.43]	0.003
Sex	Male	212.9	16	11		
	Female	423.5	26	39	0.74 [0.40,1.36]	0.33
Age (in years)	---	---	---	---	1.03 [1.01,1.05]	0.004
CAA before HIV-seroconversion (in pg/mL)	---	---	---	---	1.00 [0.99,1.00]	0.97

Figure 1 (Cumulative Incidence Function) illustrates endpoint differences between the two schistosome infection groups. The overall outcome incidence was significantly lower in HIV-1 seroconverters infected with *Schistosoma* spp. compared to those non infected with *Schistosoma* spp. (Subdistribution Hazard Ratio (SHR) = 0.31 [0.12,0.84], p = 0.021).

Figure 1 - Cumulative Incidence Function of the composite outcome CD4$^+$ T-cell counts <350 cells/µL and/or death, controlling for ART initiation, by schistosome infection status at time of HIV-1 seroconversion. Time in years.
The curve represents the cumulative incidence of the composite endpoint while controlling for the competing risk.



Attending a clinic was significantly associated with a higher rate of the outcome (SHR = 4.09[1.60,10.43], p = 0.003) and all regressions included this variable to account for the effect of missing follow-up clinical data. The rate of the outcome also differed significantly before and after the last demographic surveillance visit and before and after the last clinic visit at which the seroconverters were seen (SHR = 4.14 [1.86, 9.24], p = 0.001 and 0.31[0.14,0.71], p = 0.005, respectively, by univariable analysis). The results of the univariable analyses are presented in **Table 2**.

After stepwise analysis, the final multivariable model included schistosome infection status, CAA value pre-HIV-seroconversion, age and time of demographic surveillance visit. The impact of schistosome infection on time to outcome was still statistically significant and protective: there was an 82% reduction in risk of reaching a CD4 count <350 or death in HIV-1 seroconverters infected with schistosomes compared to those free of schistosomes (SHR = 0.18[0.068,0.50], p = 0.001) at time of seroconversion. The results of the final model are presented in **Table 3**.

Table 3 - Results of the multivariable competing risk regression based on sub-distribution hazard ratios, including control for missing observations and time-dependent covariates, with variables selected by stepwise analysis and model goodness of fit tested for (N=169).

	Sub-Hazard Ratio	95% CI	p-value
***Schistosoma* spp.-infected (ref=CAA negative at HIV-1 seroconversion)**	0.18	[0.068,0.50]	0.001
After seen at a demographic surveillance visit	4.77	[1.67,13.6]	0.003
Age at seroconversion (in years)	1.079	[1.042,1.12]	<0.001
CAA before HIV-seroconversion (in pg/mL)	1.00	[1.00,1.00]	0.004

The sensitivity analysis considered all 75 seroconverters who were lost-to-follow-up before 15th March 2017, as having reached the endpoint. The analysis included 116 occurrences of the outcome and showed a SHR for schistosome infection in the same direction but not statistically significant (SHR = 0.63[0.34,1.15], p = 0.133). When running the analysis for the less stringent definition of schistosome infection, with schistosome positivity at HIV-1 seroconversion defined as having a pre-HIV-seroconversion schistosome positive test, we observed similar results (SHR = 0.60[0.30,1.23], p = 0.16 before adjustment and SHR = 0.44[0.20,0.99], p = 0.047 after adjusting for time-dependent variables, age, and CAA before HIV-seroconversion).

DISCUSSION

Our study demonstrates that people with schistosome infection at the time of HIV-seroconversion develop adverse HIV outcomes more slowly than those without. Ours is the first study, to our knowledge, that used a longitudinal design to examine the impact of schistosome infection on HIV outcome. Because routine screening and treatment for schistosomiasis was not the standard of care during the follow-up period, our testing of banked samples provides a rare window into the long-term effects of schistosome infection on HIV-1 disease progression. Although our prior work found that HIV-1 seroconverters with schistosome co-infection develop higher HIV-1 viral load set-points[3] and would thus be expected to have more rapid HIV-1 disease progression[14, 25], our current longitudinal findings suggest more complex interactions between HIV-1 and schistosome co-infections that may be modulated over time.

Even if HIV-1 viral load set-point is indeed higher in those with HIV-1 schistosome co-infection[3], our long-term follow-up raises the question of whether HIV-1 viral load may later become lower in those with schistosome co-infection than in those with HIV-1 alone. Longitudinal studies in macaques have shown nonsignificant trends in this direction[26, 27], but human studies have yielded mixed results, some of which differ by helminth species. In support of this concept, several observational studies in patients with chronic HIV-1 infection have demonstrated higher CD4 counts and/or lower HIV-1 viral loads in those with versus those without helminth co-infection[28, 29]. Others have reported transitory increases in HIV-1 viral loads following treatment of schistosome infections[28, 30–32] or no difference in CD4 counts[33, 34] and plasma viral load[30,34] between patients with HIV-1/schistosome coinfection and HIV-1 alone. A randomized controlled trial found that providing empiric anti-helminth treatment to Kenyan adults with HIV-1 infection did not delay HIV-1 disease progression[35].

One possible explanation for our findings could be a protective effect of host immune responses to schistosomes against HIV-1 progression. Individuals with chronic schistosome infection have increased peripheral blood percentages and absolute numbers of Th17 cells and T regulatory cells as compared to uninfected individuals, particularly when they have a high degree of schistosome-induced tissue pathology[36–41]. In addition, studies in HIV-infected patients suggest that Th17 and T reg cells, as well as their ratio, may play a critical role in determining the speed of HIV/AIDS progression[42, 43]. Lower Th17/Treg ratios have been associated with more advanced HIV-1 infection[44], while absolute increases in T reg numbers have been associated with decreased markers of immune activation[44, 45], potentially leading to better HIV-1 outcomes. In addition, HIV-1 so-called “elite controllers”, who maintain very low HIV-1 viral loads and high CD4 counts without antiretroviral therapy, have been found to have higher baseline numbers of Th17 cells than other HIV-1-infected individuals, possibly because more Th17 cells could prevent microbial translocation and thereby decrease immune hyperactivation[43, 44]. Taken together, this body of evidence suggests that one possible immunologic mechanism to explain our study’s findings could be the induction of Th17 and T reg cells by chronic schistosome infection, leading to delayed HIV-1 disease progression.

Age did not modify HIV-1 disease progression, contrary to what previous studies had shown[46, 47], likely due to homogeneity in age between the two groups. It is surprising that sex was not significantly associated with HIV/AIDS progression given that studies demonstrate higher HIV-1 viral load set-points in men than in women[48–50]. It is possible that other variables were so strongly associated with the outcome that the sex effect became relatively inconsequential, that sex differences in linkage to care were small in our study, or that the complexities of interactions between host sex and schistosome infection[51] make detection of a simple relationship difficult.

Our results are to be interpreted in light of some limitations. The sero-surveys were conducted every 3 years, which only allowed us to approximate seroconversion dates and to assume that schistosome infection status at seroconversion was correlated to the infection status at the last HIV-1 negative sero-survey and first HIV-1 positive sero-survey. We were also unable to test for viral loads, or additional immunologic markers that might provide insight into the reasons for our observations, due to insufficient quantity of blood in DBS. We also assumed that people not found at a clinic did not go to an HIV clinic, which is not necessarily true, especially for people who moved from the study area shortly after seroconversion. If individuals with schistosome infection tended to be more mobile or to attend clinics outside of our catchment area, fewer of them would have reached the CD4 endpoint and some deaths could have been missed. Finally, we identified ART use as the most important competing risk that could have impacted CD4 counts and mortality, but it is possible that we did not account for other important competing risks. The sensitivity analysis considering lost-to-follow-ups as dead did not yield statistically significant results, suggesting that the analysis is sensitive to differential loss to follow up. However our assumption that all lost-to-follow-ups died is quite extreme, and the fact that the SHR yields a result in the same direction, with a p-value of 0.13, reinforces the idea that schistosome infection is associated with decreased incidence of negative HIV/AIDS outcomes.

In conclusion, our study suggests that schistosome infection at the time of HIV-1 acquisition may delay HIV-1 disease progression. Complementary findings from a variety of other studies of HIV-1/helminth co-infections strengthen the likelihood that this result is not spurious. Plausible mechanisms by which schistosome infection could delay HIV-1 disease progression include induction of Th17 or T reg cells or disrupting the Th17/Treg ratio. This work highlights the need for additional studies to examine these immunological interactions between the two pathogens on a longer-term scale.

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CHAPTER 4: IMPACT OF *SCHISTOSOMA* INFECTION ON HIV TRANSMISSION TO SEXUAL PARTNERS

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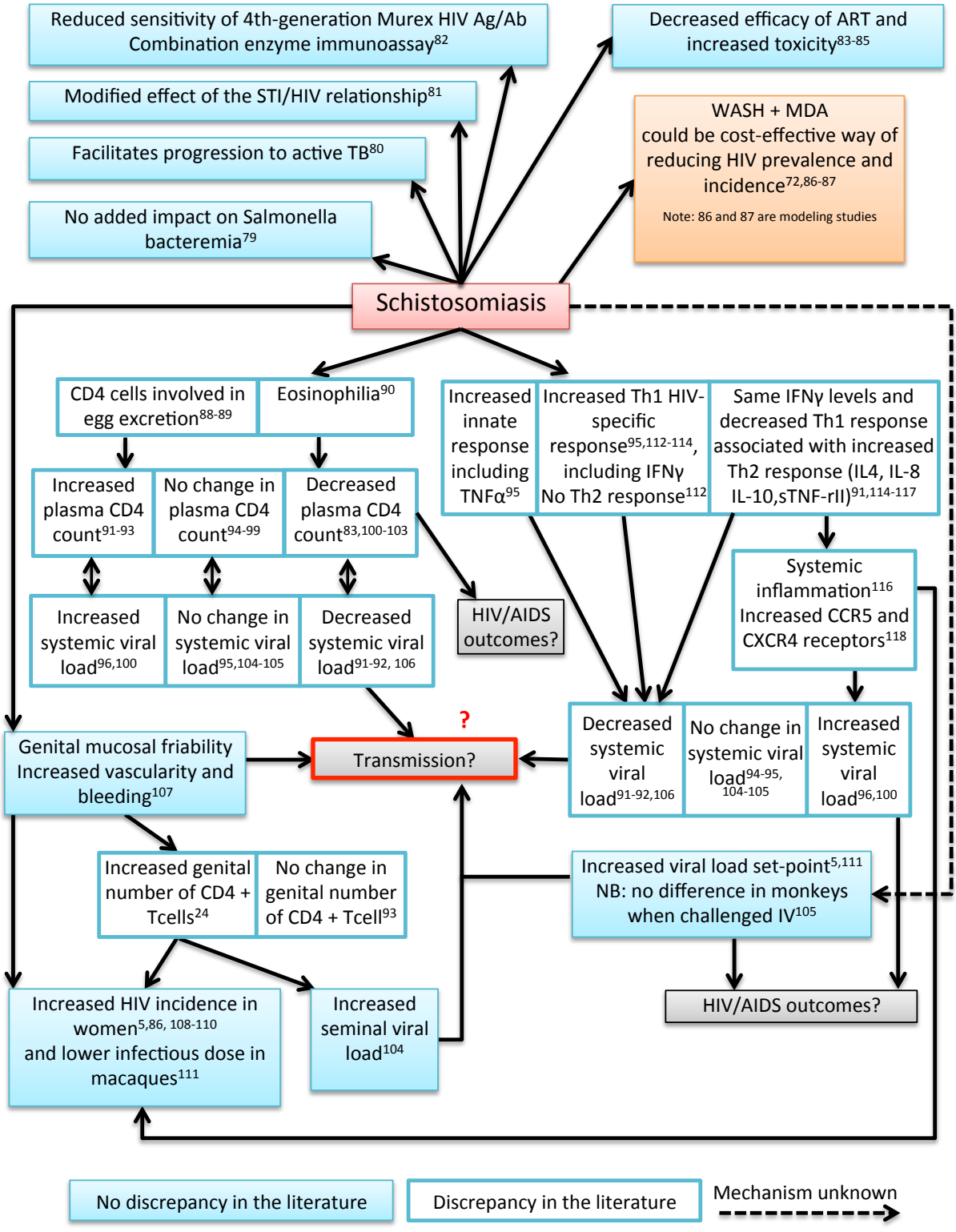
WHERE DOES THIS CHAPTER FIT?



ABSTRACT

Heterosexual transmission is the main driver of the HIV epidemic in Tanzania. Only one estimate of the incidence rate of intra-marital HIV seroconversion in Tanzania has been reported and was derived from data collected between 1991 and 1995. Moreover, little is known about the specific risk factors for intra-marital seroconversion in Tanzania, including infection with *Schistosoma* spp. Improved evidence around factors that increase the risk of HIV transmission to a serodiscordant spouse is needed to develop and improve evidence-based interventions. We sought to investigate the rate of intra-marital HIV seroconversion among HIV sero-discordant couples in Tanzania as well as its associated risk factors, including infection with *Schistosoma* spp.. We identified all HIV positive individuals in the TAZAMA HIV-serosurvey cohort and followed up their serodiscordant spouse from 2006 to 2016. The rate of seroconversion was analyzed by survival analysis using non-parametric regressions with exponential distribution. We found 105 serodiscordant couples, 14 of which had seroconverting spouse. The overall HIV-1 incidence rate among spouses of people with HIV-1 infection was 38.0 per 1000 person/years [22.5-64.1]. Notably, the HIV-1 incidence rate among HIV-1 seronegative male spouse was 6.7[0.9-47.5] per 1000 person/years, compared to 59.3 [34.4-102.1] per 1000 person/years among female spouse. After adjusting the model for schistosome status of the baseline HIV positive individual and sex of the serodiscordant spouse, female spouses had a rate of seroconversion 8.06[1.04-62.60] times higher than male spouses (p=0.046), while schistosome status of the baseline individual was not significant (adjusted hazard ratio=1.17[0.37-3.69], p=0.789). Our study suggests that schistosome infection in HIV-1 infected individuals only slightly affects HIV-1 transmission to that person's spouse. More studies are needed to look at the link between schistosome/HIV-1 co-infection in individuals and HIV-1 transmission to sexual partners, particularly in women.

Keywords: HIV, serodiscordant, intra-marriage transmission, Tanzania, schistosomiasis

INTRODUCTION

Schistosoma sp. and HIV are co-endemic globally and an estimated 6 million individuals are co-infected worldwide[1]. *Schistosoma* sp. infection has been shown to interact with HIV-1 by modifying susceptibility to the virus and impacting AIDS outcome.

Women have both increased odds and increased incidence of HIV-1 infection when *Schistosoma* co-infected[2–7]. Schistosome eggs in the mucosal tissue of the vagina and cervix trigger local physical and immunological changes that have been hypothesized to increase susceptibility to the virus during sexual HIV-1 exposure[8–11]. In addition, schistosome infection may facilitate transmission of HIV-1 to sexual partners. This could occur through exposure to blood or through higher genital tract HIV-1 RNA viral loads in the setting of genital inflammation. Schistosome eggs have been associated with genital mucosal friability and post-coital bleeding in women [12] and a recent small study of men who were co-infected with *S. haematobium* and HIV-1 documented that treatment for *S. haematobium* decreased the HIV-1 RNA viral load in semen[13].

Schistosoma sp. modifies HIV-1 RNA viral load as well. In both men and women, co-infection with *Schistosoma* sp. leads to higher plasma viral load[14–16]. Some studies have also demonstrated that treatment of *Schistosoma mansoni* is associated with a decrease in HIV-1 viral load in individuals with HIV-1-*S. mansoni* co-infection[17,18]. The increase in HIV-1 viral load in individuals co-infected with *Schistosoma* sp. could reflect the ability of schistosome infection to increase HIV-1 transmission to sexual partners[19,20].

HIV-1 RNA viral load set-points have also been found to be higher in both men and women who were infected with schistosomiasis at time of HIV-1 seroconversion[4]. HIV-1 RNA viral load set-points are highly predictive of transmission to sexual partners[21–23]. Finally, infection with *Schistosoma* sp. at time of HIV-1 seroconversion affects HIV-1 survival, with longer survival time and fewer symptoms, likely affecting sexual behavior and thus HIV-1 transmission[24].

We had a unique opportunity to investigate the impact of schistosome-HIV-1 co-infection on the rate of HIV-1 seroconversion within married couples in an ongoing HIV-seroincidence study of 30,000 people in northwest Tanzania. Our goal was to identify all HIV-seroconverters within this cohort within a 10-year time-period and to determine whether there appeared to be an increased rate of HIV transmission or acquisition among those with schistosome infection. We hypothesized that people co-infected by *Schistosoma* sp. and HIV-1 would be twice as likely to transmit HIV-1 to their sexual partners as people infected with HIV-1 alone, even after controlling for sex and sexual behavior.

METHODS

Identification of HIV-1 infected individuals

Our study was conducted within the ongoing TAZAMA project, a community-based longitudinal open HIV-testing cohort in Kisesa, northwest Tanzania, which documents detailed demographic, sexual, and behavioral data and collects dried blood spots (DBS) approximately every three years from a population of ~30,000 individuals. Those wishing to know their HIV status may undergo voluntary HIV testing and counseling on the same day as collection of the DBS. The HIV testing (sero-survey) is nested within a Demographic Surveillance System (DSS) which visits every household in the catchment area approximately every nine months to document household members and relationships. Additional details have been previously described[25]. Details collected from both the DSS and sero-surveys included the start and end dates of sexual relationships with both the spouse with whom they lived and with external sexual partners and the frequency of sexual intercourse.

For this project, we identified all individuals whose DBS tested positive for HIV-1 or who were found to be HIV-1 positive at an HIV testing clinic within the TAZAMA cohort between 2006 and 2013. Throughout the rest of the methods we will refer to these individuals as "baseline individuals" for clarity and brevity.

Identification of serodiscordant spouse and relationship time period

Through the DSS we identified all spouses of baseline individuals and obtained their HIV-1 test results from both the sero-surveys and from HIV tests at other clinics. We excluded couples that were never serodiscordant from the analysis, and couples for which the spouse had HIV-1 seroconverted more than 6 months after either partner reported the end of the relationship. For each couple, we determined the at-risk dates for HIV-1 seroconversion during which they reported being in a sexual relationship with a partner who was HIV-1 positive. We collected demographic and sexual behavior data from the first DSS or sero-survey following the start of the serodiscordant relationship. Sexual behavior data included the number of extra-marital partners, having sex with sex workers, and traveling men. Condoms are rarely used within the context of marriage in Tanzania[26, 27].
We used data from all sero-surveys until the last sero-survey with questions pertaining to the relationship time period. Seroconverters were defined as individuals who had been HIV-1 seronegative in one sero-survey and who were found to be HIV-1 seropositive in a subsequent sero-survey. All DBS available until the date of the spouse potential seroconversion were tested for Schistosoma circulating anodic antigen for both the baseline individual and his or her spouse.

Follow-up

The follow-up period started either from the start of the relationship or from the first positive HIV result for the baseline individual. The follow-up period ended either at the spouse's seroconversion date, or at the end of the relationship, or at the last sero-survey for which a spouse had an available HIV-1 test result and remained HIV-seronegative. The seroconversion date was approximated as the mid-point between the last negative DBS and the first positive test, either at a sero-survey or at another clinic.

Schistosome infection status

We measured schistosome Circulating Anodic Antigen (CAA) in banked DBS to determine schistosome infection status. The CAA test is a genus-specific assay that detects a gut-associated antigen secreted into the host bloodstream by adult schistosome worms. The test does not differentiate between the Schistosoma mansoni and haematobium species present in the Kisesa area of Tanzania. We defined schistosome positivity during the relationship as having at least one positive test for schistosome infection in all DBS available during the follow-up period. Both schistosome positivity as a binary variable and worm load defined as the natural logarithm of CAA were used in the analysis.

Laboratory testing

Dried blood spots - DBS were collected by finger prick onto a Whatman Protein Saver 903 card (GE Healthcare Bio-Sciences, Pittsburgh, PA). DBS cards were dried out of direct sunlight and sealed in a gas-impermeable zip bag with desiccant and humidity indicator. Cards were stored at the National Institute for Medical Research (NIMR) laboratory in Mwanza at -20°C.

HIV-1 testing - Diagnosis of HIV-1 infection was confirmed using a screening and subsequent confirmatory test, as recommended by national HIV guidelines, at each time point. These were: Uniform II Category III Ab test followed by Enzygnost test (sero-surveys 5 and 6), Uniform II Category IV Ab+Ag test followed by Enzygnost test (sero-survey 7), and Determine test followed by Unigold test (sero-survey 8). Samples that were negative at the screening test were reported as negative. Samples that were positive at the screening test were tested with the confirmatory test. If the confirmatory test was negative, the final result was reported as negative. If the confirmatory test was positive, the final result was reported as positive.

Schistosoma *sp. testing* - DBS were tested for schistosome by CAA at Leiden University Medical Center by eluting whole blood from DBS and then concentrating the sample as previously described[28]. A lower limit threshold of 2 pg CAA per mL of eluted blood was used for the assay. 35 individuals had stored serum samples but no DBS samples available for testing and underwent serum CAA testing at NIMR with a lower limit threshold of 30 pg CAA per mL[29]. Samples scoring values above the threshold were designated positive for *Schistosoma* infection.

Statistical analysis

Analysis included all couples as described above. Binary variables were described as proportions and continuous variables were described using median and interquartile range. We assessed differences in baseline characteristics using Chi-square or Fisher's exact test for proportions and the nonparametric equality test for medians.

A survival analysis was conducted to investigate the difference in HIV-1 seroconversion rates between spouse of a baseline individual co-infected versus not co-infected with Schistosoma. The event of interest was defined as HIV-1 seroconversion. Data was censored at the end of the relationship or for loss to follow-up, defined as the last negative sero-survey at which the spouse provided a DBS. The Kaplan-Meyer method was used to compare time to seroconversion between the HIV-serodiscordant spouses of baseline individuals who did and did not have schistosome infection. A non-parametric regression with exponential distribution, adjusted for all significantly different baseline factors as well as biologically sound variables, was used to assess endpoint incidence difference by schistosome infection status. Time-dependent variables characterized at each sero-survey (such as number of extra-marital sex partners, frequency of sex, etc) were defined as representative of the time period following the sero-survey and are called "survey-dependent variables" for the rest of the manuscript.

Variables that were associated with failure at 10% significance were individually included into the model and model goodness-of-fit assessed through step-wise analysis. Based on the results of the analysis, a second analysis was performed after stratifying by sex of the serodiscordant spouse. All analyses were performed in STATA 14.1 (College Station, TX, USA). All results were expressed with 95% confidence intervals (CIs) and statistical significance was set at $P < 0.05$ (two-tailed).

A sensitivity analysis was conducted where schistosome infection was considered a survey-dependent variable. We conducted a second sensitivity analysis in which survey-dependent variables were defined as representative of the time period preceding the sero-survey results. Finally we conducted a third sensitivity analysis in which we excluded all couples for which the baseline individual was on ART.

Ethical considerations

Ethical approval for retrospective and prospective analysis of these data was obtained from Bugando Medical Centre in Mwanza (BREC/001/04/2011), the National Institute for Medical Research in Dar es Salaam (NIMR/HQ/R.8a/Vol.IX/2446), and Weill Cornell Medicine in New York (1108011883). Study participants provided consent during enrollment into the cohort study as per the approved procedures of the TAZAMA project, which included consent for future testing of DBS samples[25].

RESULTS

We identified 1439 baseline individuals who were found to be HIV-1 seropositive at a sero-survey between 2006 and 2012. Of these 554 had at least one spouse registered in the DSS after the time of the baseline individual's first positive test. Among the 554, 289 had at least one spouse who had HIV-1 test results and 105/289 were serodiscordant couples between 2006 and 2015 who met criteria for inclusion in this analysis. From these serodiscordant couples, this yielded 368.8 years of total analysis time at risk and under observation.

63.8% (67/105) of couples had a male baseline individual and a female serodiscordant spouse. An overwhelming proportion of baseline individuals and spouses were of Sukuma ethnicity (97.1% (102/105) and 92.4% (97/105), respectively), Christian (83.8% (88/105) and 92.4% (87/105), respectively), and reported having only one spouse (87.6% (92/105) and 90.5% (95/105), respectively). 52.2% (48/92) of the baseline individuals were schistosome positive. 54.5% (55/101) of the serodiscordant spouses were schistosome positive. All couples were heterosexual. The demographics of the population are presented in **Table 1** as a comparison between the 14 people who HIV-seroconverted during follow-up and the 91 people who did not. Serosurvey-dependent variables are presented in **Table 2**.

Table 1 - Characteristics of the spouse, baseline individual and couple by spouse seroconversion status.

Variable	Non-seroconverters N=91	Seroconverters N=14	p-value
Variables concerning the baseline individual			
Schistosome CAA positivity	51.3% (41/80)	58.3% (7/12)	0.647
Sex (Female)	40.7% (37/91)	7.1% (1/14)	0.016
Education (Received at least one year of formal schooling)	24.4% (22/90)	0.0% (0/14)	0.037
ART intake	12.1% (11/91)	0% (0/14)	0.353
Marital status (Polygamy)	13.2% (12/91)	7.1% (1/14)	1
Age in years at the start of the time period of interest	39[33-45]	44[37-53]	0.125
Variables concerning the serodiscordant spouse			
Schistosome CAA positivity	54.0% (47/87)	57.1% (8/14)	0.828
Sex (Female)	59.3% (54/91)	92.9% (13/14)	0.016
Education (Received at least one year of formal schooling)	42.9% (39/91)	21.4% (3/14)	0.037
Marital status (Polygamy)	12.1% (11/91)	0% (0/14)	0.353
Age in years at the start of the time period of interest	37[31-46]	35.5[32-46]	0.828
Male and circumcised	46.4% (13/28)	--	--
Variables concerning the couple			
Age difference between the baseline individual and his/her spouse	-3[-9;4]	-5[-8;-4]	0.246
Length of the time period of interest (in days)	1029 [691-1882]	1093.5[571-1150.5]	0.228

Table 2 - Results of the univariable analysis for factors associated with HIV-1 seroconversion.

Variable		Person-time (in years)	Number of events	HR [95%CI]	p-value
Variables concerning the baseline individual					
Schistosome CAA positivity	Negative	167.80	5		
	Positive	172.27	7	1.35[0.43-4.24]	0.611
Sex	Male	221.50	13		
	Female	149.43	1	0.11 [0.015-0.87]	0.036
Education	Never attended school	294.91	14		
	Ever attended school	71.01	0	0[0]*	0.992
ART intake	No	318.72	14		
	Yes	50.06	0	0[0]*	0.992
Ln(CAA)#	---	-----	---------	1.18 [0.93-1.49]	0.177
STI symptoms	No	291.31	11		
	Yes	77.46	3	1.03[0.29-3.68]	0.969
Variables concerning the serodiscordant spouse					
Schistosome CAA positivity	Negative	152.33	6		
	Positive	196.75	8	1.03[0.36-2.98]	0.953
Sex	Male	149.43	1		
	Female	221.50	13	8.77[1.15-67.04]	0.036
Education	Never attended school	234.99	11		
	Ever attended school	135.94	3	0.48[0.13-1.72]	0.258
Other risks for HIV#**	No	297.77	14		
	Yes	71.00	0	0[0]*	0.994
Risky sex behavior^#	No	66.43	4		
	Yes	19.15	0	0[0]*	0.994

Ln(CAA) [#]	--	---	---	1.11[0.85-1.44]	0.453
Number of extramarital partners[#]	None	303.95	14		
	One or more	66.97	0	0[0]*	0.994
STI Symptoms[#]	No	285.8	12		
	Yes	82.94	2	0.57[0.13-2.57]	0.468
	Variables concerning the couple				
Age difference between the baseline individual and his/her spouse in years	---	---	---	1.00[0.9991-1.001]	0.734
Sex frequency[#]	Less than once a month	37.22	1		
	Between once a month and once a week	134.64	6	1.66[0.20-13.78]	0.639
	More than once a week	166.50	5	1.12[0.13-9.57]	0.919

**No convergence of the model due to presence of zeros. No conclusion on the association between the variable and seroconversion can be made due to short person-time available. ART was still included in the final model stepwise analysis.*

***Other risks for HIV include incisions and transfusions*

^Risky sex behaviors include having sex with women at bars or with traveling men.

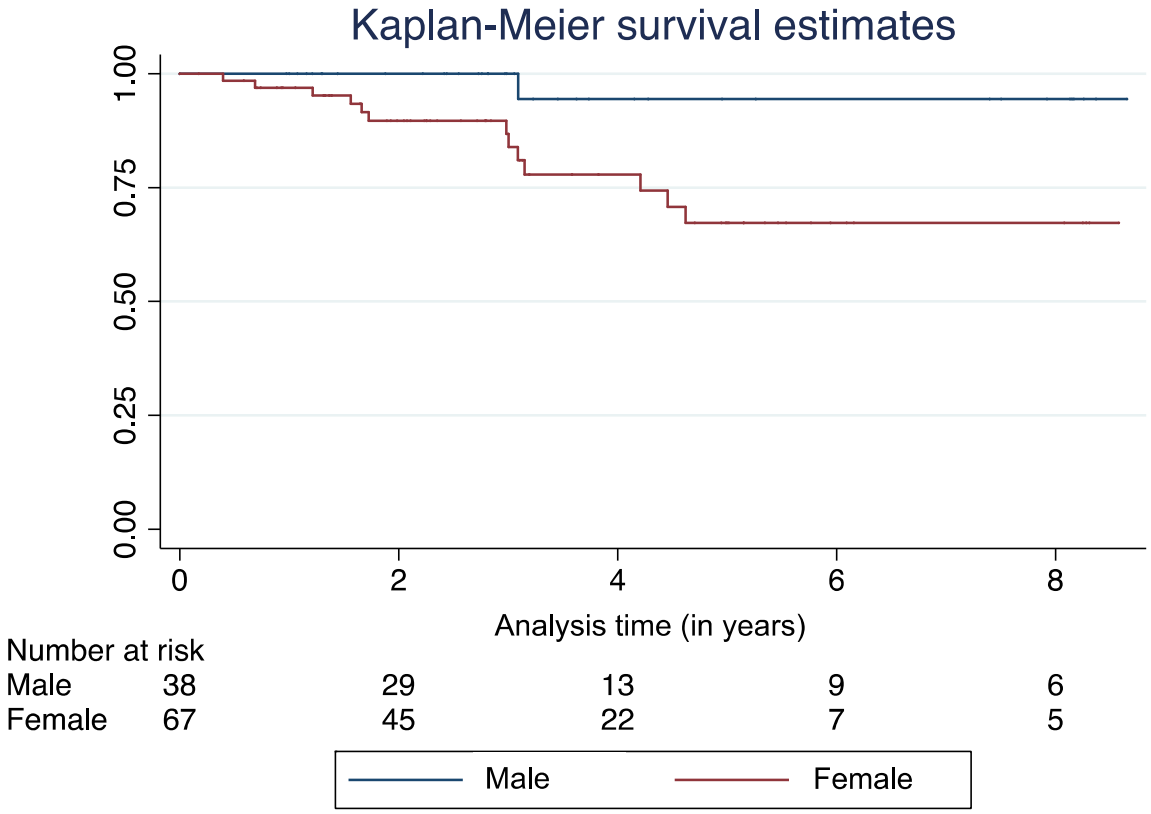
#Survey-dependent variables

14/105 (13.3%) partners HIV-1 seroconverted, and 13 of these were women. The overall HIV-1 incidence rate among spouses of people with HIV-1 infection was 38.0 per 1000 person/years [22.5-64.1]. Notably, the HIV-1 incidence rate among HIV-1 seronegative male spouse was 6.7[0.9-47.5] per 1000 person/years, compared to 59.3 [34.4-102.1] per 1000 person/years among female spouse.

After univariable analysis, schistosome status of the baseline individual was associated with an increase in rate of spouse seroconversion, although not significant (Hazard Ratio (HR)= 1.35[0.43-4.24], p=0.611 for the variable "schistosome positivity", and HR=1.18[0.93-1.49], p=0.177 for the variable ln(CAA+1), as a continuous, survey-dependent, variable). Sex of the serodiscordant partner was the only variable that was significantly associated with HIV-1 seroconversion. Specifically, HIV-1 uninfected female spouses of HIV-1 infected male baseline individuals were found to have higher incidence rates of seroconversion than HIV-1 uninfected male spouses of HIV-1 infected female baseline individuals (HR=8.77, p=0.036). Results of the univariable analyses are presented in **Table 2**.

After stepwise multivariable analysis, sex of the serodiscordant partner was the only variable that yielded a best of fit model, even when forcing the variable "schistosome status of the baseline individual" into the model. After adjusting the model for schistosome status of the baseline individual and sex of the serodiscordant spouse, female spouses had a rate of seroconversion 8.06[1.04-62.60] times higher than male spouses (p=0.046), while schistosome status of the baseline individual was not significant (adjHR=1.17[0.37-3.69], p=0.789). After stratification by sex, schistosome status of the baseline individual was still not significant for female spouses (HR=1.41[0.41-4.83], p=0.582). The Kaplan-Meyer survival curves by spouse sex are presented in **Figure 1**.

Figure 1 - Kaplan-Meier survival estimates for seroconversion by sex of the spouse.
The curve represents the risk of seroconverting over time by sex of the serodiscordant spouse.



All sensitivity analyses led to similar results and are presented in **Table 3**.

Table 3 - Results of the sensitivity analyses.

Sensitivity analysis	Variable	adjHR[95%CI]	p-value
Schistosomiasis as a survey-dependent variable	Schistosome CAA positivity in the baseline individual	2.08[0.61-7.12]	0.24
	Sex of the serodiscordant spouse	7.65[0.98-59.76]	0.052
Excluding ART from the analysis	Schistosome CAA positivity in the baseline individual	1.22[0.39-3.84]	0.74
	Sex of the serodiscordant spouse	8.32[1.07-64.43]	0.043
Survey dependent variables representative of the time period preceding the survey results	Schistosome CAA positivity in the baseline individual	1.96[0.33-11.75]	0.46
	Sex of the serodiscordant spouse	0[0]	0.99

DISCUSSION

In this in-depth study of a community of approximately 30,000 individuals, the intra-marriage HIV-incidence in our study population was overall 19 times the general national HIV-incidence[30]. This effect was largely due to women being highly susceptible to incident HIV infection, yielding an incidence of 60 seroconversions per 1000 person-years in women and only 7 per 1000 person-years in men. This is a greater than eight-fold increase in HIV acquisition in women as compared to men, and far higher than observed elsewhere in Africa. Our results suggest that intra-marriage seroconversion in serodiscordant couples deserves more attention in Tanzania, and that disproportionate transmission from men to women, particularly in the absence of female-controlled HIV-prevention measures, may continue to push the HIV epidemic towards female predominance.

To our knowledge, our study is the first to look at the role of schistosome infection in the transmitting partner on HIV-1 incidence and to give an estimate of the hazard ratio of HIV-transmission from schistosome co-infected transmitting partners compared to non-co-infected transmitting partners. It shows a directional trend towards an increased transmission of HIV-1 from baseline individuals infected with *Schistosom*a spp. . This could be due to genital schistosomiasis caused by both *S. haematobium* and *S. mansoni* in men and women[8, 31]. The local physical damage done to the mucosal barrier might make *Schistosoma*-infected individuals more prone to transmitting HIV during intercourse[12, 32, 33]. In addition, genital schistosomiasis triggers inflammation and increased genital vascularity, which in turn leads to the accumulation of CD4 cells, higher local shedding and facilitated transmission of HIV virions[11, 15, 34, 35].

Notably, despite a wide confidence interval, our point estimate for the increased hazard of HIV transmission is rather small. The fact that CAA also led to a hazard ratio close to 1 does suggest that schistosome positivity of the transmitting partner might play only a small role in transmission of HIV-1. Our previous study looking at the impact of schistosome infection on HIV/AIDS outcomes also showed surprising results of a protective effect of schistosome infection at time of HIV-1 seroconversion on HIV-1 disease progression[24]. Both studies align with the possibility that despite higher viral load set points in schistosome positive individuals at time of HIV-1 seroconversion[4, 15], there might be some immunological long term changes that decrease the viral load, possibly to lower values than seen in those with HIV infection alone. Several other studies support this possibility, documenting no difference in viral load among co-infected versus non- co-infected people[13, 36, 37], transitory higher viral loads after praziquantel treatment[38, 39], or lower viral loads in co-infected individuals[40].

It is also possible that the clinical effect is not as strong as expected due to the schistosome species involved. While the eggs of both *S. mansoni* and *S. haematobium* can lead to genital lesions, *S. mansoni* eggs accumulate in genital tissue at lower concentrations and therefore are less likely to create such lesions[8, 41, 42]. Our Kisesa cohort is more likely to be infected with *S. mansoni* than *S. haematobium*[2, 6, 28, 43]. It is thus possible that despite being infected with schistosomes, the baseline individuals were infected with *S. mansoni* and had low

prevalence of genital schistosomiasis and/or a lower density of egg-induced local changes that may facilitate HIV transmission.
More surprisingly, the schistosome status of the receiving partner did not appear to have a major impact on HIV-seroconversion here[2–5]. It is biologically possible that sex of the receiving partner was so strongly associated with HIV-1 seroconversion[44] that any other risk factor for transmission became relatively inconsequential. Women are indeed more at risk of HIV-1 infection than men, as widely recognized in the literature[44, 45].

The usual risk factors for HIV-1 seroconversion within marriage, such as sex frequency, count of extra-marital sex partners and age difference with the spouse, were not significant within our cohort either[45, 46]. It is possible that this was due to the higher age of our study participants than has been observed in most other serodiscordant couple studies[47]. In these older adults, traditional HIV risk factors may be less important. Our results are consistent with the well-described finding that, per sex act, women are indeed more at risk of HIV-1 infection than men,[44] likely due to a larger surface area of the vagina and the ability of the virus to pass easily through the cells of the vaginal lining. Too few partners were on ART to assess the role of ART on seroconversion in our study, although the finding that no partners of baseline individuals on ART seroconverted is consistent with other studies that showed lower incidence of HIV when HIV positive partner was on ART.

Our results are to be interpreted in light of some limitations. Our study had only enough power (80%) to detect significance for a hazard ratio of 3.7 or above. To detect significance for a hazard ratio of 1.5, we would have needed approximately 2250 person-years in each schistosome infection group, or a total of 4500 person-years, which is more than 10 times the follow-up of our study. As a result, we cannot conclude on the statistical significance of our findings regarding schistosome infection, and our confidence intervals are large. More studies with a larger number of HIV-1 serodiscordant couples are needed to obtain a better estimate of the hazard ratio of HIV-transmission from schistosome positive/HIV-1 positive individuals compared to from schistosome negative/HIV-1 positive individuals. In addition, studies conducted in regions with higher prevalence of *S. haematobium* infection may more easily detect a significant difference in hazard ratios for those with versus without infection.

We were unable to test for viral loads, or additional immunologic markers that might provide insight into the reasons for our observations, due to insufficient quantity of blood in DBS. We were also unable to perform phylogenetic analyses that would have permitted determination of whether the HIV-seroconverting partner had been infected from a partner outside of the marriage. Partners might also under or overestimate the number of extra-marital sexual partners based on their gender[48]. Despite those limitations, our finding that all sensitivity analyses gave the same results strengthens confidence in the quality and accuracy of our analysis.

In conclusion, our study suggests that schistosome infection in HIV-1 infected individuals only slightly affects HIV-1 transmission to that person's spouse. More studies are needed to look at the link between schistosome/HIV-1 co-infection in individuals and HIV-1 transmission to sexual partners, particularly in women.

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CHAPTER 5 - HIV-1 VIRAL LOADS ARE NOT ELEVATED IN INDIVIDUALS CO-INFECTED WITH *SCHISTOSOMA* SPP. AFTER ADJUSTMENT FOR DURATION OF HIV-1 INFECTION

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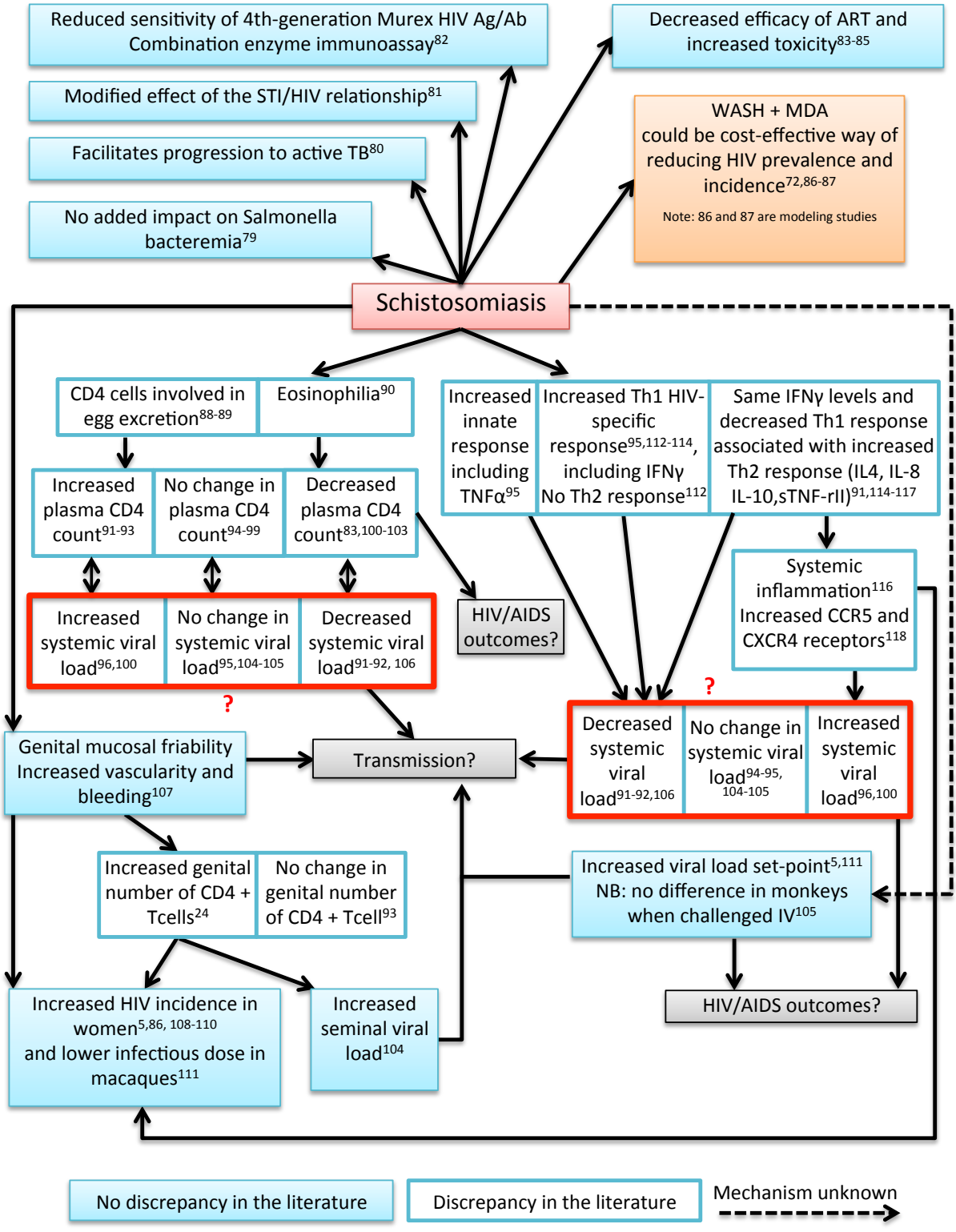
WHERE DOES THIS CHAPTER FIT?



ABSTRACT

Studies of the role of *Schistosoma* co-infections on plasma HIV-1 RNA (HIV-1 viral load) have yielded incongruent results. The role of duration of HIV-1 infection on the link between *Schistosoma* and HIV-1 viral load has not been previously investigated. We aimed to assess the impact of HIV-1/*Schistosoma* co-infections on viral load in Antiretroviral Treatment (ART)-naïve HIV-1 infected people taking into account the duration of HIV-1 infection. We describe 79 HIV-infected outpatients greater than 18 years of age who had never used ART in Mwanza, Tanzania. Schistosomiasis testing was done by urine and stool microscopy and by serum *Schistosoma* circulating anodic antigen (CAA) testing. *Schistosoma* positivity was defined as having either test positive. We conducted univariable and multivariable linear regressions to assess the relationship between *Schistosoma* infection and the $\log_{10}$ of viral load. Duration of HIV infection was calculated using the first measured $CD4^+$ T-cell (CD4) count as a function of normal CD4 count decay per calendar year in drug naïve individuals. An active *Schistosoma* infection was demonstrated in 46.8% of the patients. The median $\log_{10}$ viral load was 4.5[3.4–4.9] $\log_{10}$ copies/mL in *Schistosoma* uninfected patients and 4.3[3.7–4.6] $\log_{10}$ copies/mL in *Schistosoma* infected patients. *Schistosoma* co-infection was negatively associated with the $\log_{10}$ of viral load after adjustment for *Schistosoma* intensity as measured by CAA, CD4 counts at time of testing, and duration of HIV-1 infection ($\beta = -0.7[-1.3;-0.1]$, $p = 0.022$). *Schistosoma* co-infection was not associated with viral load in univariable analysis. There was also no interaction between *Schistosoma*positivity and duration of HIV-1 infection. Our study is the first, to our knowledge, to report adjustment for duration of HIV-1 infection when analyzing the relationship between HIV-1 viral load and *Schistosoma* spp. We found that time infected with HIV-1 has a major effect on the relationship between HIV-1 viral load and *Schistosoma* infection and may be a critical explanatory factor in the disparate findings of studies on HIV-1 viral load and schistosomiasis. The $\log_{10}$ viral load difference found indicates that *Schistosoma* co-infection does not make HIV progression worse, and could possibly lead to slower HIV disease progression.

Keywords: *Schistosoma* spp., HIV-1, Viral load, Plasma HIV-1 RNA, Tanzania

INTRODUCTION

Although Africa makes up just 15% of the worldwide population, it is burdened by 70% of the world's 36.7 million HIV infections and 91% of the world's 240 million *Schistosoma* infections[1, 2]. The 2013 Global Burden of Disease Study estimated that schistosomiasis alone causes 2.6 million disability-adjusted life years (DALYs) lost annually, while HIV infection alone causes 66.7 million DALYs[3]. Of note, DALY calculations for HIV and schistosomiasis account for each infection separately and do not consider impacts that they may have on one another.

A growing body of animal and human studies supports a complex relationship between HIV and schistosomiasis. Animal studies suggest that schistosomiasis may alter immune control of viral co-infections, facilitating viral reactivation and replication[4–6]. However the role of *Schistosoma* spp. co-infections on plasma HIV-1 RNA (HIV-1 viral load) is still unclear, with various studies reporting higher, lower, or equivalent HIV-1 viral loads in those with *Schistosoma* co-infection[7–15]. Within this body of evidence, the longest time that people with HIV and *Schistosoma* co-infection have been followed was approximately 24 months. Our group has recently documented an unexpected improved long-term HIV disease-free survival in those with HIV/*Schistosoma* co-infections at time of HIV-1 seroconversion[16]. This suggests that chronic *Schistosoma* infection may downregulate HIV-1 viral replication even though the opposite has been observed during acute infection[6, 15], or that time infected with HIV may have been a confounder in studies that examine the link between *Schistosoma* spp. and HIV-1 viral load.

We thus aimed to assess the impact of HIV-1/*Schistosoma* spp. co-infections on viral load in Antiretroviral Treatment (ART)-naïve HIV-1 infected people taking into account the duration of HIV-1 infection. To investigate this question, we designed a study situated within an outpatient HIV clinic at which approximately 30% of individuals are *Schistosoma* -infected[17, 18], and enrolled patients who would shortly be starting ART.

METHODS

Study Participants and Enrollment

This study was conducted in April and May 2015 in an HIV outpatient clinic at Bugando Medical Centre (BMC) in Mwanza. The participants were HIV-infected adults greater than 18 years of age who had never used ART according to clinic records and patient report.

Eligible patients provided a single urine and stool sample for schistosomiasis testing by microscopy in order to determine which species of schistosomes were present, as well as serum for quantitation of *Schistosoma* Circulating Anodic Antigen (CAA), which measures the intensity of infection[19]. Plasma was also collected for viral load measurement. Additional information was extracted from the HIV clinic database and the patient's chart.

Laboratory Methods

Microscopic testing was performed on 10 mL of urine by the filtration technique and on feces following the Kato Katz method. Testing was performed by parasitologists at the National Institute of Medical Research (NIMR) in Mwanza, Tanzania. Five Kato Katz slides using 41.7 mg of stool per slide were used, which has been shown to have a sensitivity comparable to collecting three stool samples on different days[20]. CAA testing was performed at NIMR in Mwanza as previously described, using a positivity threshold of 30 pg/mL (dry reagent SCAA20 assay format)[19]. In order to maximize sensitivity of testing for *Schistosoma* infections in this HIV-infected population, we used a composite score in which we defined *Schistosoma* infection as having either a microscopy or CAA positive test. Plasma viral load was quantified using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (Roche Molecular Systems Inc., Pleasanton, California, USA) at the BMC clinical laboratory, with a lower limit of detection of 20 copies/mL.

Statistical Analysis

Data was double entered, verified and cleaned using Microsoft Excel 2013 and analysis was performed using STATA version 13. Categorical data were described with proportions and continuous data were described with median and interquartile range. Chi-square tests and t-tests were used to compare presence of demographic and clinical factors between those co-infected with *Schistosoma*/HIV-1 and those infected with HIV-1 only. Both viral loads and CAA values had extreme outliers and were skewed to the right. We thus used the log10 of viral load and natural log of CAA, by convention. Univariable and multivariable linear regressions were used to assess the relationship between *Schistosoma* infection and the log10 of viral load. We also assessed the association between *Schistosoma* infection and CD4 counts. All variables significantly associated with the outcome in the univariable analysis were included in the multivariable analysis. A stepwise analysis was conducted for the multivariable analysis. A quantile regression was used to assess the interaction between

Schistosoma infection and duration of HIV-1 infection and its impact on the difference in median of the log10 of viral load.

Time infected with HIV was defined using the first CD4+ T-cell counts (CD4 counts) reported at the clinic. This method has been previously used with some variation[21–25]. Due to similarity in the available data and study setting, we used the method of Forbi et al.[23]. The CD4 counts at time of enrollment at the clinic were used to approximate the time delay between HIV infection and enrollment as a function of normal CD4 decay per calendar year in drug naïve individuals. The normal reference values of CD4 counts in healthy Tanzanians have been estimated at a median of 596.5 [291.2–1278.9] cells/μL for men and 764.5 [288.5–1406.8] cells/μL for women[26]. In addition, the most prevalent HIV infecting clade in the Lake Zone in Tanzania is clade A[27] and in Mwanza, Tanzania, Clade A and D viruses make up the majority (34 and 28% respectively) of HIV infections[28]. This is similar to proportions found on the Ugandan side of the lake, in Rakai[29] and Entebbe[30] and the overall normal CD4 decay per calendar year, across all clades, has been shown to be approximately 34.5 cells/μL per year in Rakai[29].

Starting from the upper range of the normal reference values for CD4 counts, we modeled decay by the square-root function as suggested by Kiwanuka et al.[29], which meant we subtracted 5.87 cells1/2/μL1/2 from 35.76 cells1/2/μL1/2 for men and 37.51 cells1/2/μL1/2 for women per calendar year period until the square root of the first CD4 count reported at the clinic was reached. The time period for this to happen was considered to be the estimated period between HIV-1 acquisition and enrollment at the clinic. The time between the first CD4 count reported at the clinic and the date of viral load testing was then added to this variable to obtain the duration of HIV-1 infection. This led to an estimated median time from seroconversion to enrollment of 2.5[1.7–3.0] years, which is similar to the median time from seroconversion to enrollment estimated by our group within the Kisesa Lake region cohort using mid-dates between two serosurveys as date of seroconversion (manuscript submitted). Finally, to look at the interaction between *Schistosoma* infection and time infected with HIV, we categorized the latter using tertiles.

Ethical Considerations

All participants were recruited after providing written informed consent in accordance with the declaration of Helsinki. Clearance was obtained from the joint CUHAS/BMC Research Ethics Committee, the National Institute for Medical Research in Dar es Salaam, Tanzania, and Weill Cornell Medical College, New York. All clinical data were made available immediately to clinicians and recorded in the patient's medical record. All patients with *Schistosoma* infection received praziquantel 40 mg/kg free of charge.

RESULTS

We enrolled 83 HIV-infected patients who presented at the clinic and had never initiated ART. Thirty-seven out of seventy-nine (46.8%) were positive for *Schistosoma* spp. either by CAA or microscopy test. 33/81 (40.7%) were positive by CAA and the median CAA was 18.3[5.6–517.2] pg/mL. The distribution of CAA values was skewed to the right. Therefore we log-transformed it and the median ln CAA was 3.0[1.9–6.3] ln pg/mL. None had a positive urine microcopy and among those with positive stool microscopy (20/81–24.7%), the median of the mean egg count was 21.6[4.8–52.8] eggs/gram. Seventeen patients were CAA positive but urine and stool negative, while 4 patients were stool positive but CAA negative. Patients had a median age of 36[29-41] years, and 67/83 (80.7%) were female. Median CD4 counts at enrollment was 504[395-749] cells/μL and median CD4 counts at time of viral load testing was 455[328-614] cells/μL.

Patients had enrolled in the HIV clinic a median of 2.5[1.7–3.0] years after acquiring HIV, and provided viral loads for this study a median of 3.7[3.0–5.7] (minimum = 1.7, maximum = 12.0) years after acquiring HIV. The median viral load was 21,670.5[2,852.0–56,160.0] copies/mL, or 4.3[3.5–4.7] log10 copies/mL. The median log10 viral load was 4.5[3.4–4.9] log10 copies/mL in *Schistosoma* uninfected patients and 4.3[3.7–4.6] log10 copies/mL in *Schistosoma* infected patients. The main variables are presented in **Table 1** by *Schistosoma* infection status.

After univariable linear regression, female sex, higher CD4 counts and longer time infected with HIV-1, were all significantly associated with lower log10 of the viral load. *Schistosoma* positivity and ln of CAA were not associated with log10 of the viral load (**Table 2**).

Our unadjusted data shows a typical relationship between viral load and time infected with HIV-1, as described by other studies (**Figure 1A**)[31].

Table 1 - Characteristics of the 79 HIV-1 infected patients tested for *Schistosoma* infection who had never initiated ART.

	***Schistosoma* free (n=42) n/N(%) or median[IQR]**	***Schistosoma* infected (n=37) n/N(%) or median[IQR]**	**p-value**
Female sex	37/42 (88.1%)	27/37 (73.0%)	0.087
CD4 count at time of VL testing (cells/µl)	475 [314.5-658.5]	439 [336-566]	0.66
Log_{10} of viral load (copies/mL)	4.5[3.4-4.9]	4.3[3.7-4.6]	0.21
Age in years	36 [28-42]	35 [30-40]	0.81
Years infected with HIV-1 (as a continuous variable) *	3.7 [3.0-6.5]	3.7 [3.0-5.3]	0.72
Years infected with HIV-1*			0.82
<3 years	12/40 (31.6%)	12/36 (30.3%)	
3-5 years	13/40 (28.9%)	13/36 (39.4%)	
>5 years	15/40 (39.5%)	11/36 (30.3%)	

**3 patients did not have any CD4 count reported, precluding calculation of the years infected with HIV.*

Table 2 - Results of the univariable analysis with log_{10} of viral load as a continuous outcome.

	Slope coefficient (95%CI)	**p-value**
Sex (Female)	-0.6 [-1.1;-0.08]	0.024
CD4 count at time of VL testing		
<200 cells/µl	Ref	
200-500 cells/µl	-0.7 [-1.3;-0.02]	0.042
500-1000 cells/µl	-1.2 [-1.9;-0.5]	0.001
>1000 cells/µl	-1.06 [-2.0;-0.1]	0.024
***Schistosoma* positivity**	-0.15 [-0.6;0.3]	0.49
Age (in years)	0.02 [-0.005 ; 0.04]	0.13
Ln of CAA in ln pg/mL	0.006 [-0.07;0.08]	0.86
Years infected with HIV-1	-0.1[-0.2;-0.03]	0.008

Figure 1 - Relationship between log10 of the viral load and time infected with HIV (A) Unadjusted, (B) Adjusted for Schistosoma status, ln of CAA and CD4 counts.

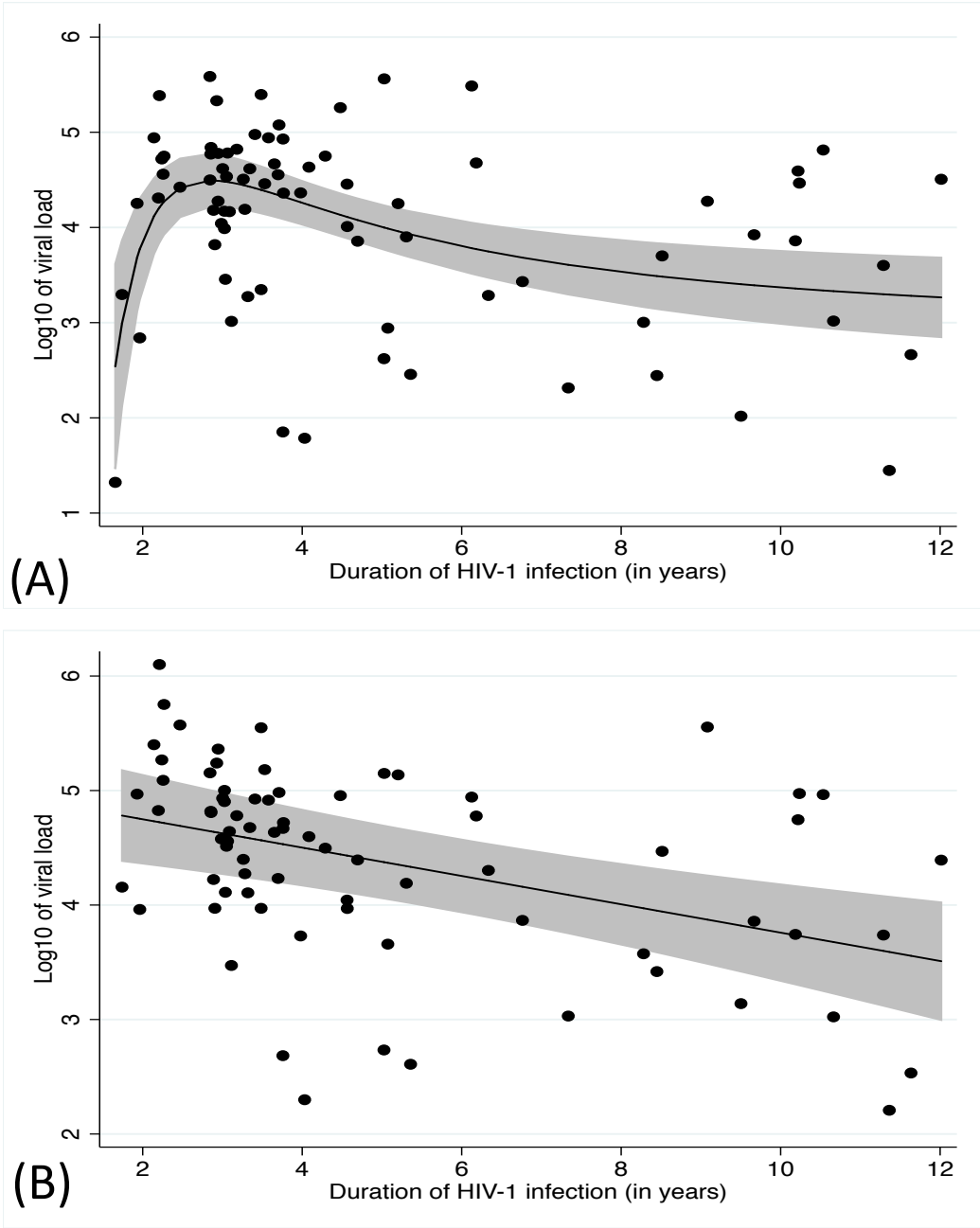


Figure (A) shows the crude relationship between log10 of viral load and duration of HIV-1 infection. A fractional polynomial was fitted to the data. The black line represents the predicted log10 of viral load after applying the resulting function to the data. The grey area represents the 95% confidence limits around the fitted values. The black dots represent the residuals. Figure (B) shows the relationship between log10 of viral load and duration of HIV-1 infection after adjustment for Schistosoma infection status, ln of CAA and CD4 counts using a fractional polynomial. The black line represents the predicted log10 of viral load after applying the resulting function to the data. The grey area represents the 95% confidence limits around the fitted values. The black dots represent the residuals.

After stepwise multivariable analysis, the best-fit model for log10 viral load included *Schistosoma* positivity, ln of CAA, CD4 counts at time of study enrollment and time infected with HIV-1. *Schistosoma* positivity was negatively associated with the log10 of viral load after adjustment (−0.7[−1.3;−0.1], p = 0.022). Sex and age were not part of the best-fit model. The best-fit model is presented in **Table 3**. Of note, this model includes both the *Schistosoma* infection status as a binary variable and also the natural log of the CAA value to assess whether the intensity of the *Schistosoma* infection impacted the viral load. The relationship between duration of HIV-1 infection and log10 of viral load adjusted for *Schistosoma* status, ln of CAA, and CD4 counts is shown in **Figure 1B**.

Table 3 - Results of the multivariable linear regression with log_{10} of viral load as a continuous outcome.

	Slope coefficient (95%CI)	**p-value**
***Schistosoma*-infection status**	-0.7 [-1.3;-0.1]	0.022
Ln of CAA in ln pg/mL	0.08 [-0.007;0.2]	0.070
CD4 counts at VL testing		
<200 cells/µl	Ref	
200-500 cells/µl	-0.7[-1.2;-0.1]	0.022
500-1000 cells/µl	-1.2[-1.8;-0.6]	<0.001
>1000 cells/µl	-1.7[-2.7;-0.7]	0.001
Years infected with HIV-1	-0.1[-0.2;-0.06]	<0.001

When looking at whether the difference in median log10 of viral load between *Schistosoma* infected and uninfected patients changed over time, we found no statistical significance. **Figure 2** assesses the difference in median of log10 viral load between *Schistosoma* infected and uninfected patients within each HIV-1 infected time category.

Figure 2 - Comparison between median viral loads by time infected with HIV-1 and by *Schistosoma*-infection status.

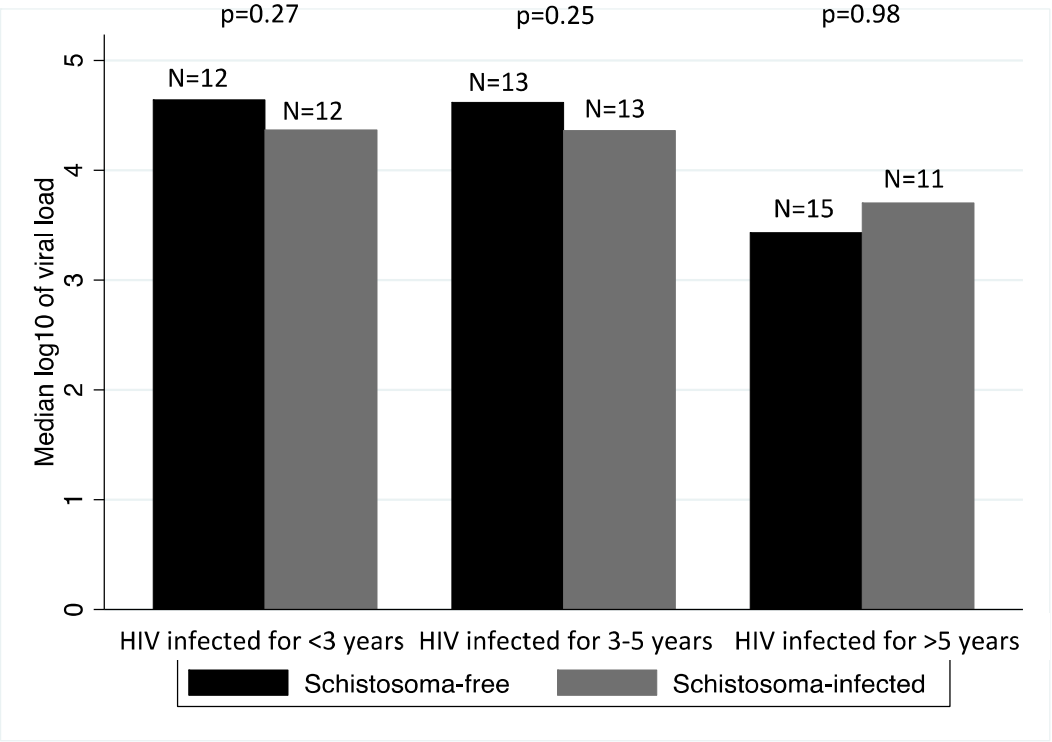


Figure 2 shows the median log_{10} of viral load in Schistosoma *-free and* Schistosoma *-infected patients, by category of duration of HIV-1 infection. The difference in median log_{10} of viral load was assessed by rank-sum test. There was no difference in median log_{10} of viral load between* Schistosoma *-free and* Schistosoma *-infected, regardless of the duration of HIV-1 infection.*

We assessed the impact of time on the difference in median of log10 viral load between *Schistosoma* infected and uninfected patients using a quantile regression with an interaction term between *Schistosoma* status and duration of HIV-1 infection. In other words, we assessed whether the difference in viral loads between *Schistosoma* infected and uninfected patients seen in people HIV infected for 3–5 years is significantly different from the difference seen in people HIV infected for <3 years, and again for >5 vs. <3 years. The quantile regression showed no significant interactions between *Schistosoma* status and time infected with HIV-1.

In our cohort, *Schistosoma* status was not associated with CD4 counts (slope coefficient = −48.2 [−184.8; 88.4], p = 0.48).

DISCUSSION

Our study showed that current infection with *Schistosoma* spp. was associated with significantly lower HIV-1 viral loads after adjusting for CD4 counts and time infected with HIV-1. The viral load difference of $0.7\log_{10}$ copies/mL would be expected to lead to over a 60% decrease in risk of HIV transmission and of reaching AIDS-related death for *Schistosoma* -co-infected patients compared to *Schistosoma* -free patients[32]. This is in close alignment with the 82% decrease in risk of reaching lower CD4 counts and/or death found by our group[16] using a different analysis technique and in a different population. Taken together, these findings suggest the possibility that long-term HIV outcomes may be positively affected by *Schistosoma* infection.

Our study is the first, to our knowledge, to report adjusting for duration of HIV-1 infection when studying the relationship between HIV-1 viral load and *Schistosoma* spp. Time infected with HIV-1 was a main confounder of the relationship between HIV-1 viral load and *Schistosoma* infection. It is well known that CD4 counts and viral load in HIV-1 infected individuals, and the rates at which they change, differ over time[33–35], which makes it difficult to compare changes in these parameters between two individuals at different periods of their HIV-1 infection[36]. Duration of HIV-1 infection may thus be a critical explanatory factor in the disparate findings of studies on HIV-1 viral load and *Schistosoma* infections[7–15], as suggested by Walson et al.[36]. Other studies' lack of control for duration of HIV-1 infection may have hindered accurate analysis of the relationship between HIV-1 and *Schistosoma* infections. This may also be true for most studies looking at the relationship between other helminths and HIV-1 infections.

In addition, the control for ART initiation has been inconsistent in most studies. Many investigators have either assumed that all participants were ART naïve due to past limited availability of ART, or have not mentioned ART intake at all in their studies[7, 8, 10, 11, 15]. Importantly, given the drastic effect of ART on CD4 counts and HIV-1 viral load[31], failure to account for ART intake in even a few individuals could have biased the results. By choosing patients who had been followed in the HIV outpatient clinic and would be beginning ART in the near future, and by measuring their viral load before initiation of ART, we avoided having the relationship between *Schistosoma* spp. and HIV-1 viral load distorted by the effect of ART on viral loads.

The association between sex and viral load on univariable analysis is unsurprising. Male sex has previously been shown to be associated with higher viral loads[37–39]. The decrease in viral load with increasing CD4 counts has also been previously documented in sub-Saharan Africa[40, 41]. The overall decrease in viral load over time infected with HIV-1 is logical, as too few of our patients have been infected with HIV-1 for over 10 years to see the late-stage re-increase in viral loads.

Our results are to be interpreted in light of some limitations. The sample size was small due to the expense and relative unavailability of viral load testing at a time when viral load testing was just becoming available at our clinic. In addition, despite not being significant in our

study or in a study in South Africa[42], others have shown an impact of *Schistosoma* spp on CD4 counts[8, 10, 11, 16, 43–45], potentially biasing our calculation of the duration of HIV infection. Assessing this relationship using other methods to determine the length of HIV infection would be useful. Nonetheless, the facts that significance is still attained and that variables expected to impact viral load do impact it strengthen confidence in the quality and accuracy of our analysis. Larger, longitudinal studies would be useful in order to investigate the potential interactions between duration of HIV-1 infection and *Schistosoma* infection and its effect on HIV-1 viral load. Investigating the effect of praziquantel treatment would also be of interest given studies that suggest that tissue lesions may not regress after treatment[46].

In conclusion, our work demonstrates that individuals with HIV-1 and *Schistosoma* co-infections had lower viral loads than those with HIV-1 alone, when accounting for time infected with HIV-1. The difference in viral load suggests that *Schistosoma* infection may not lead to worse HIV-1 outcomes nor higher HIV-1 transmission. Future studies of interactions between HIV-1 and *Schistosoma* spp. should account for the duration of HIV-1 infection in their analyses.

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CHAPTER 6: DECREASED SENSITIVITY OF *SCHISTOSOMA* SP. EGG MICROSCOPY IN WOMEN AND HIV-INFECTED INDIVIDUALS

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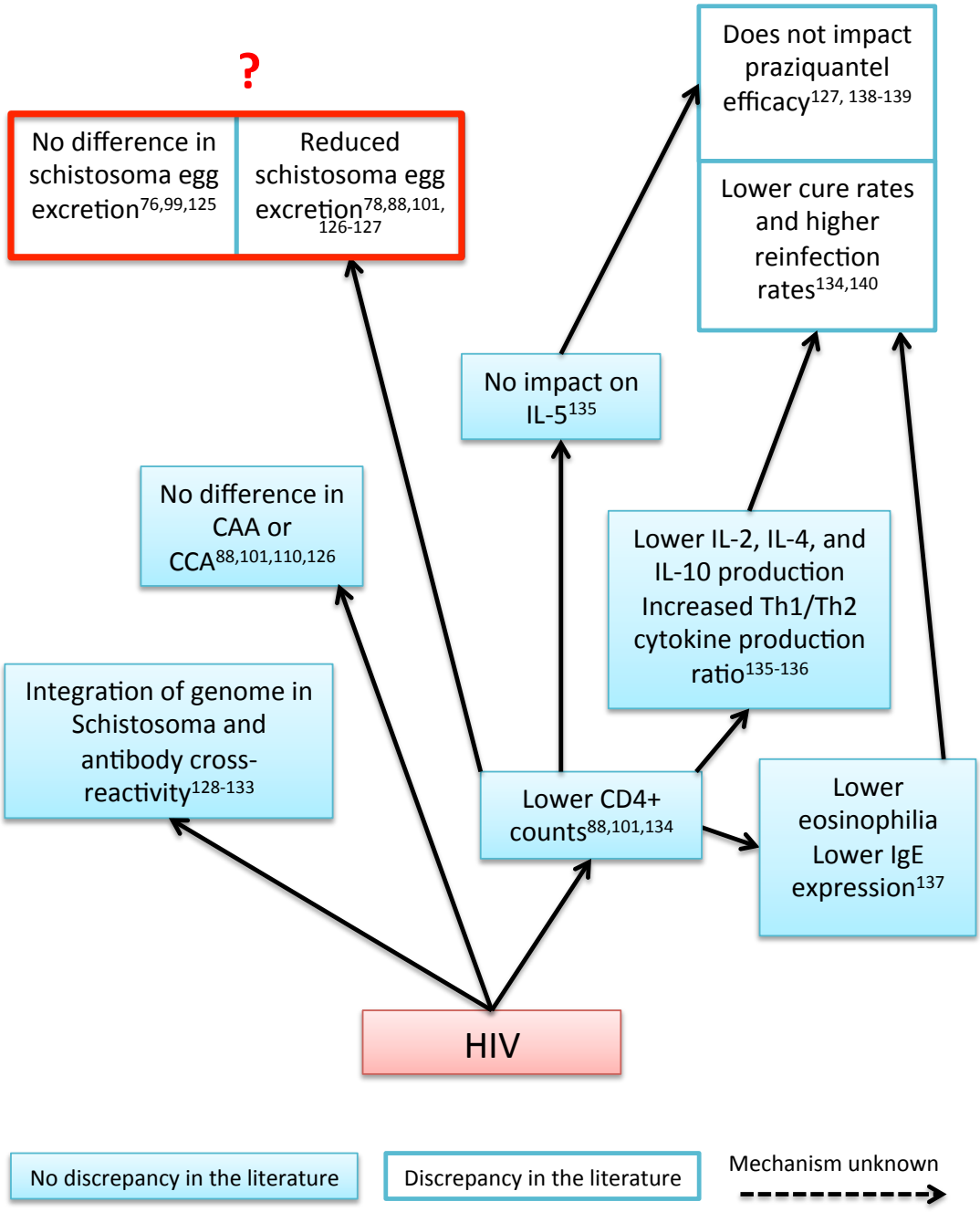
WHERE DOES THIS CHAPTER FIT?



ABSTRACT

It has been postulated that impaired host immunity due to HIV infection reduces parasite egg excretion. *Schistosoma*/HIV interactions have also been shown to differ by sex. We hypothesized that egg excretion would vary based on both HIV status and sex. We examined data from over 1700 participants in 8 studies conducted in northwest Tanzania between 2010 and 2016. *Schistosoma* infection was defined by Circulating Anodic Antigen (CAA) serum levels ≥30 pg/mL and/or egg positivity in either stool by Kato Katz method or urine by filtration. We used multivariable analyses to determine the impact of confounding factors such as sex, age, previous praziquantel treatment, and worm burden as measured by serum CAA level, on the relationship between egg excretion and HIV status. HIV-infected individuals were significantly less likely to excrete schistosome eggs than HIV-uninfected individuals, even after controlling for worm burden and sex (OR=0.6[0.4,0.9], p=0.005). Furthermore, after controlling for worm burden and HIV status, women had lower odds of egg excretion than men (OR=0.4[0.3,0.5], p<0.001). Sensitivity of egg microscopy was lower in HIV-infected than HIV-uninfected men (41% versus 61%, p<0.001), while sensitivity in women remained low in both groups (33% versus 37%, p=0.664). Our study is the first to report that women with *Schistosoma* infection excrete fewer eggs than men for a given worm burden, regardless of HIV status. These findings suggest that guidelines for use of microscopy to diagnose *Schistosoma* infections in HIV-infected individuals and in women merit reconsideration.

Keywords: Schistosoma sp., HIV, microscopy, Circulating Anodic Antigen

INTRODUCTION

Schistosomiasis is a zoonotic neglected tropical disease with a life cycle through fresh water snails that affects 218 million individuals worldwide[1]. For endemic settings the World Health Organization recommends microscopic examination of stool and urine for parasite eggs to detect *Schistosoma* infections[1]. However, egg excretion is variable, depends on both the worm load and the host immunity, and can fluctuate on a daily basis[2-5]. Microscopy is known to have a low sensitivity in areas of low endemicity and in individuals with light infections[6-8].

With the development of an up-converting phosphor lateral flow assay to measure *Schistosoma* Circulating Anodic Antigen (CAA), a newer technique has emerged with higher sensitivity and better specificity[9-11]. CAA is a glycosaminoglycan-like carbohydrate that is secreted into the bloodstream by adult worms of all *Schistosoma* species and can be used to estimate the burden of adult worms[4, 7, 12, 13]. Studies of *Schistosoma* infections that have used both CAA and microscopy have consistently revealed non-negligible numbers of patients who are CAA-positive while having a null egg count[14-20].

Discordant findings of a CAA test positive for *Schistosoma* antigen but no eggs visualized microscopically may occur more commonly in the setting of HIV infection, which is co-endemic with *Schistosoma* infection in many regions of sub-Saharan Africa. Mouse models suggest that intact T cell responses may be necessary for efficient parasite egg excretion[21], supporting field-based observations in humans[22, 23]. Several small studies have reported lower *Schistosoma* egg excretion in those with HIV infection as compared to those without[22,24-26], while larger studies have not been able to show an association[27-29]. Only one study looked at CAA values in relation to HIV status and found no difference[30]. None of these studies has investigated whether the sex of the infected host affects differential results between CAA and egg microscopy.

We sought to determine the effect of HIV infection on *Schistosoma* egg excretion using CAA and microscopy data from a total of eight different studies and screening projects conducted by our team in northern Tanzania[31, 32], and to investigate this relationship in both *S. mansoni* and *S. haematobium* infection. We hypothesized that HIV-infected individuals with *Schistosoma* infection were significantly less likely to shed eggs than those without HIV infection. We also hypothesized that the ratio of egg to CAA values, reflecting eggs excreted for a given worm burden, would differ by HIV status and that egg excretion may differ by sex.

METHODS

We compiled data from eight cross-sectional studies conducted in northwest Tanzania from 2010 to 2016, two of which had their methods previously described[31, 32]. Altogether, the eight studies covered 20 villages of the Lake Zone. In all studies, individuals over 18 years old were enrolled after providing written informed consent. All included participants underwent testing for *Schistosoma* infection both by egg count in stool and urine and by serum CAA. HIV status was determined by rapid test on site in accordance with the Tanzanian national algorithm for HIV testing at the time of the study, with all positive results confirmed by a second different rapid test. Those testing positive for HIV infection for the first time were referred to their local clinics for ongoing free care and treatment. All *Schistosoma* infections were treated with praziquantel. Basic demographic data was collected including age, sex, and treatment for schistosomiasis within the last 5 years. Details of the individual studies are presented in **Table 1**.

Microscopy testing was performed on 10mL of urine (for *S. haematobium*) by the filtration technique and on feces (for *S. mansoni*) following the Kato-Katz method. Testing was performed on site by the same experienced parasitologists from the National Institute of Medical Research (NIMR) in Mwanza, Tanzania for all studies. For study A, two Kato Katz slides were prepared from each stool sample using 41.7 mg of stool per slide, while 5 Kato Katz slides using 41.7 mg of stool per slide were used for all other studies. Serum CAA testing was performed at Leiden University Medical Center for Study A, and the remaining CAA testing was performed at the National Institute for Medical Research in Mwanza as previously described, using a positivity threshold of 30 pg/mL (dry reagent SCAA20 assay format)[10, 33] for all studies. Species were determined by egg morphology. In the case of CAA positive, egg-negative cases, *Schistosoma* species cannot be identified, but as the epidemiological distribution of both species of *Schistosoma* was known for all villages, the most likely species was assigned in monospecies villages.

Informed consent was obtained from all participants. All studies received ethical approval from Bugando Medical Centre, the National Institute for Medical Research in Dar es Salaam, Tanzania, and Weill Cornell Medical College, New York, USA.

Statistical analysis was performed using Stata version 13 (College Station, TX, USA). Individuals were defined as *Schistosoma* -infected if they had a serum CAA concentration of ≥30 pg/mL and/or *Schistosoma* eggs detected by microscopy. Binary variables were described as proportions and continuous variables were described using median and interquartile range and compared using chi-squared tests. We investigated the association between HIV status and egg excretion, regardless of *Schistosoma* species, by running univariate logistic regressions for the subgroup "*Schistosoma* -infected" with the outcome being presence of eggs in urine and/or feces and input being HIV status.
We further ran multivariable analyses to explore the impact of other competing factors such as sex, age, previous praziquantel treatment for schistosomiasis, and worm load, on the relationship between egg excretion and HIV status. HIV status did not take into account the

duration of HIV infection or the use of antiretroviral therapy (ART). The natural logarithm of CAA values was used as a proxy for worm load[4, 12]. Variables included in the final model were determined by backward selection procedure with the standard threshold of 0.1 as well as exploration of interaction terms.

In addition, we explored CAA values by sex and HIV status. Finally we examined the relationship between the CAA and egg load, by *Schistosoma* species due to large differences in excretion numbers, with regard to sex and HIV status by running rank-sum tests on egg/CAA ratios.

RESULTS

In total, results were available from 1745 participants tested in 20 villages near Lake Victoria. 54.6% (953/1745) of them were female, 52.3% (913/1745) of them were positive for *Schistosoma* infection, 22.5% (393/1745) were HIV-infected and 18.1% (256/1413) reported treatment for schistosomiasis in the past 5 years (**Table 1**).

Table 1 - Baseline description of the population and studies included in the analysis.

Study	N	Females	Type of study *	Median age in years [IQR]	Species **		Egg +	CAA and/or Egg+	HIV +	Egg count median [IQR]		Previous treatment ***
A	326	100.0% (326/326)	CB	30 [25-37]	*S. m.* *S. h.* Un.	57.2% (95/166) 4.8% (8/166) 38.0% (63/166)	12.0% (39/326)	50.9% (166/326)	6.1% (20/326)	Stool (/g feces) Urine (/mL urine) --	36 [24-60] 9 [5.5-12] --	17.0% (36/212)
B	82	81.7% (67/82)	OB	36 [29-40]	*S. m.* *S. h.* Un.	100.0% (38/38) 0.0% (0/38) 0.0% (0/38)	24.4% (20/82)	46.3% (38/82)	100.0% (82/82)	Stool (/g feces) Urine (/mL urine) --	21.6 [4.8-50.4] -- --	Not available
C	173	61.8% (107/173)	OB	41 [36-46]	*S. m.* *S. h.* Un.	100% (84/84) 0.0% (0/84) 0.0% (0/84)	11.6% (20/173)	48.6% (84/173)	100.0% (173/173)	Stool (/g feces) Urine (/mL urine) --	37.2 [14.4-50] -- --	2.3% (4/173)
D	668	0.0% (0/668)	CB	34 [25-42]	*S. m.* *S. h.* Un.	75.3% (321/425) 17.4% (75/425) 7.8% (31/425)	40.0% (267/668)	63.6% (425/668)	5.7% (38/668)	Stool (/g feces) Urine (/mL urine) --	48 [14.4-231.6] 6 [2-14] --	27.6% (183/663)
E	109	60.6% (66/109)	CB and OB	31 [25-40]	*S. m.* *S. h.* Un.	23.9% (11/46) 80.4% (37/46) 0.0% (0/46)	27.5% (30/109)	42.2% (46/109)	44.0% (48/109)	Stool (/g feces) Urine (/mL urine) --	55.2 [18-85.2] 4 [2-13.75] --	7.2% (7/97)
F	108	100.0% (108/108)	CB	25.5 [21-31]	*S. m.* *S. h.* Un.	2.4% (1/42) 97.6% (41/42) 0.0% (0/42)	21.3% (23/108)	38.9% (42/108)	0.0% (0/108)	Stool (/g feces) Urine (/mL urine) --	72 [72-72] 6 [4-7.75] --	Not available
G	199	100.0% (199/199)	CB	29 [23-37]	*S. m.* *S. h.* Un.	63.0% (46/73) 37.0% (27/73) 0.0% (0/73)	12.6% (25/199)	36.7% (73/199)	9.5% (19/199)	Stool (/g feces) Urine (/mL urine) --	14.4 [4.8-39] 2 [1-3.75] --	9.9% (19/191)
H	80	100.0% (80/80)	CB and OB	30.5 [25-35]	*S. m.* *S. h.* Un.	89.8% (35/39) 5.1% (2/39) 5.1% (2/39)	32.5 % (26/80)	48.8% (39/80)	16.3% (13/80)	Stool (/g feces) Urine (/mL urine) --	14.4 [4.8-103.2] 3.5 [3.25-3.75] --	9.1% (7/77)
Total	1745	54.6% (953/1745)	-	32 [25–40]	*S. m.* *S. h.* Un.	69.1% (631/913) 20.8% (190/913) 10.5% (96/913)	25.8% (450/1745)	52.3% (913/1745)	22.5% (393/1745)	Stool (/g feces) Urine (/mL urine) --	38.4 [14.4-129.6] 5 [2-12] --	18.1% (256/1413)

*CB=Community-based, OB=Outpatient clinic-based; **_S.m._=_S.mansoni_, _S.h._=_S.haematobium_, Un.=Unidentified. Only 4 individuals had known mixed infection. Individuals that were CAA+/Egg- in villages where both _S.mansoni_ and _S. haematobium_ coexist were classified as “species unidentified”.; ***This column reports treatment for schistosomiasis with praziquantel within the past 5 years.

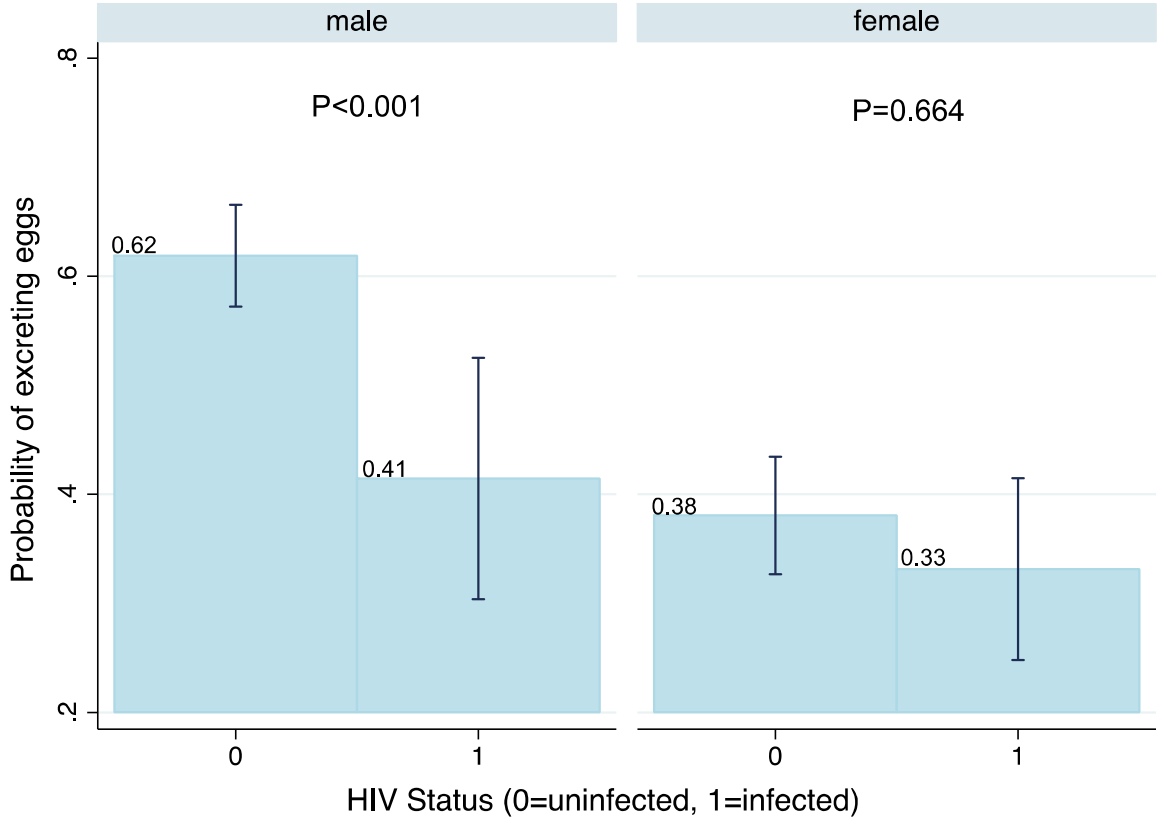
Among the 913 *Schistosoma sp.*-infected individuals, by univariate analysis, HIV infection was associated with lower odds of egg excretion (OR = 0.5 [0.4,0.7], p < 0.001) as was female sex (OR = 0.4 [0.3,0.5], p < 0.001). Past treatment with praziquantel was associated with higher odds of egg excretion (OR = 1.5 [1.1,2.2], p = 0.025), while age was not significantly associated with egg excretion (OR = 1.0 [0.98,1.01], p = 0.25). The final model determined by backward selection procedure included HIV status, sex and worm load as measured by serum CAA level. HIV remained associated with egg excretion after adjustment for sex and worm load (OR = 0.6 [0.4,0.8], p = 0.004). Of note, when adding an interaction term between HIV and sex, HIV was still significantly associated with egg positivity (OR = 0.4 [0.2,0.7], p = 0.001 for male) and the interaction between sex and HIV status was marginally significant (OR = 1.9 [0.95,3.83], p = 0.069, with male sex as the baseline), indicating that the effect of HIV infection in decreasing egg excretion was greater in males than in females.

Among *Schistosoma* -infected individuals, regardless of the infecting species and of HIV status, women were overall less likely than men to excrete eggs. In HIV-noninfected women, egg microscopy had a sensitivity of 38% and in HIV-noninfected men, egg microscopy had a sensitivity of 62% for *Schistosoma* infection (38% vs 62% difference, p < 0.001). In HIV-infected women egg microscopy had a sensitivity of 33% and in HIV-infected men egg microscopy had a sensitivity of 41% (33% vs 41% difference, p = 0.23). These results are shown in **Table 2** and the interaction margins are shown in **Figure 1**.

Table 2 - Results of the multivariable logistic regression for factors associated with *Schistosoma sp.* egg positivity.

	Variables	Odds Ratio	95% Confidence Interval	p-value
Main effects on egg positivity	HIV infection	0.6	[0.4,0.8]	0.004
	Female sex	0.4	[0.3,0.5]	< 0.001
	Serum CAA level (natural log)	1.2	[1.1,1.3]	< 0.001
With interaction allowed	HIV infection for male	0.4	[0.2,0.7]	0.001
	Female sex for HIV-uninfected	0.4	[0.3,0.5]	< 0.001
	Serum CAA level (natural log)	1.2	[1.1,1.3]	0.003
	HIV*sex (ref=male)	1.9	[1.0,3.8]	0.069

Figure 1 - Predictive margins with 95% confidence intervals for the probability of schistosome egg excretion in *Schistosoma sp* -infected individuals, by HIV status and sex.



The predictive margins probability of excreting eggs based on sex and HIV status interaction show that women are overall less likely than men to excrete eggs regardless of HIV status. Men secrete significantly fewer eggs when HIV-infected, as compared to men who are HIV-noninfected.

The median of the logarithm of CAA value did not differ by HIV status (p = 0.84) but differed by sex, with women having significantly lower CAA values (median in male = 6.6 pg/mL, median in female = 6.0 pg/mL, p < 0.001). Moreover, the ratio of eggs to CAA, reflective of the number of eggs excreted for a given worm burden, varied significantly between men and women and between HIV-infected and HIV-noninfected individuals with *S. mansoni* co-infection (p = 0.014 and p = 0.0077 respectively). Men and HIV-noninfected individuals shed more eggs for a given CAA value than did women and HIV-infected individuals. In *S. haematobium* infection, for which the sample size was much smaller, similar trends were observed but the difference did not reach significance. These results are shown in **Table 3**.

Table 3 - Median ratios and interquartile range for eggs excreted per natural log of serum CAA value, by *Schistosoma* species, sex, and HIV status.

		Schistosoma mansoni		*Schistosoma haematobium*	
		Eggs per gram stool / ln(CAA)	**P-value**	**Eggs per 10 cc urine / ln(CAA)**	**P-value**
By sex	Male	6.0 [2.3-26.5]	0.014	1.4 [0.5-2.2]	0.26
	Female	4.4 [1.6-8.6]		0.7 [0.4-1.9]	
By HIV status	Negative	6.2 [2.1-20.6]	0.0077	0.9 [0.4-2.3]	0.73
	Positive	4.1 [1.7-7.4]		1.3 [0.6-2.2]	

To determine the effect of the use of two Kato-Katz slides instead of five in study A, we conducted a sensitivity analysis that only included results from the two first Kato-Katz slides of each study. All findings remained statistically significant.

DISCUSSION

This is the first study, to our knowledge, to investigate the impact of sex on egg excretion in adults in an HIV-endemic area. Examination of serum samples from over 1700 people in Tanzania showed that both women and HIV–infected individuals were significantly less likely to excrete *Schistosoma sp.* eggs when infected, even after controlling for a given worm antigen level. The sensitivity of egg microscopy, regardless of species, was much lower in HIV-infected men than in HIV-noninfected men (41% versus 62%), while the sensitivity in HIV-infected versus HIV-noninfected women remained low in both groups (33% versus 38%). Given the marked geographical overlap of *Schistosoma sp.* and HIV infections, our work suggests that guidelines for use of microscopy to determine *Schistosoma sp.* infection status in HIV-infected individuals and in women merit reconsideration.

To our knowledge, our study is the first to report overall lower odds of egg excretion for *Schistosoma sp.* infection in women compared to men. We additionally report the novel finding that HIV infection impacts egg excretion in men but not in women[24, 25]. Only one study conducted in children reports higher *S. haematobium* egg excretion in boys than girls although this study did not control for worm burden[34]. Our finding that the ratios of egg excretion to worm burden were lower in women infected with *S. mansoni* implies that the sex difference cannot be attributed to worm burden alone for this species. It is possible that $CD4^+$ T-cell counts could have been lower in men[35, 36] and that this could have impacted egg excretion via an effect on T cells[22, 23]. It is also possible that anatomical pelvic differences between men and women could lead to higher numbers of migrating parasite eggs trapped in female pelvic tissues than in males, or that worm fecundity could be affected by disparate immunological responses to *Schistosoma mansoni* worms in men versus women[37, 38]. We had very few data points of participants both infected with HIV and *S. haematobium* and additional studies are needed to understand the effects of HIV infection on egg excretion in individuals with *S. haematobium* infection.

Our finding that HIV infection status did not significantly affect *Schistosoma sp.* egg excretion in women could explain why several larger studies, which included mostly or entirely women[27 , 28], failed to demonstrate an effect of HIV infection on egg excretion. Our study confirms the reduction in egg excretion in HIV-infected individuals that was previously observed in smaller studies[22, 24-26], even after controlling for other confounding variables. Further studies are required to look at the potential difference of old versus new HIV infection and possible impact of ART on egg excretion.

The fact that age was not associated with egg excretion seems somewhat surprising[2]. It seems likely that this finding could be a consequence of the relatively tight age range (20 to 47 years) of adults included in our study. We did not enroll children younger than age 18 and included only few adults above 40.
Finally, as could be expected, we found some CAA+/Egg- patients, which is likely because the CAA test is more sensitive than egg count. We did also identify a small number of CAA-/Egg+ patients. It is likely that CAA testing with increased sample volume, which has an

even higher sensitivity, would have identified CAA infection in some of these patients[10]. In addition, some individuals could have been recently treated with praziquantel, and due to the rapid clearance of CAA would test negative for CAA while continuing to excrete eggs[39]. Our finding that HIV status was not associated with a difference in CAA values, as shown in previous studies[30, 40], further strengthens support for use of CAA as a superior diagnostic tool for *Schistosoma sp.* infection in HIV-endemic settings and suggests that efforts to expand CAA testing are warranted.

In conclusion, our study demonstrates for the first time the effect of sex on *Schistosoma sp.* egg excretion and clarifies past studies on the relationship between HIV and egg excretion. Our work indicates that decreased egg excretion in the setting of HIV infection is limited to men. Our finding that HIV does not impact the relationship between CAA values and egg load suggests that the more sensitive CAA assay for diagnosis of *Schistosoma* infections in HIV-endemic settings, particularly for women, should be the preferred test.

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CHAPTER 7 – SUMMARIZING DISCUSSION

I. INITIAL COMPREHENSION ON *SCHISTOSOMA* SPP. AND HIV INTERACTIONS

There is a need to better understand the epidemiology of HIV and *Schistosoma* spp. co-infections, the consequences of one disease on the public health interventions targeting the other, the confounders in the interaction, and the ways to alleviate the combined burden to improve quality of life. The 2013 Global Burden of Disease Study estimated that schistosomiasis alone causes 2.6 million Disability-Adjusted Life Years (DALYs) lost annually, while HIV infection alone causes 66.7 million DALYs[1]. These numbers do not include the combined impact of co-infections. This thesis aimed at increasing the knowledge about HIV/*Schistosoma* spp. co-infections, as well as defining gaps in the past research methodology to improve future research and improve comparisons and advance accurateness of conclusions.

The studies described in this manuscript were conducted among the Tanzanian population of the Lake Zone, where regular HIV surveillance is conducted and where both *S. mansoni* and *S. haematobium* are present. *S. haematobium* and *S. mansoni* are thought to modify HIV epidemiology through both local and systemic modifications. *S. haematobium* eggs, as well as *S. mansoni* eggs to a lesser extent, can be found in the urogenital tract. They cause urogenital schistosomiasis, which is marked by inflammation, friability, and bleeding of the urinary and genital mucosa[2, 3]. Men and women are affected differently by genital schistosomiasis as genital lesions due to eggs' sequestration and induced inflammation are less common in men than in women[2, 4, 5]. In addition, sequestered eggs and their associated lesions in women are found in sites directly accessible during sexual contact[3], which is not the case for men[2, 4]. *Schistosoma* eggs are highly immunogenic and lead to recruitment of immune cells, including CD4+ T-cells (CD4 counts), that are preferential targets of HIV[2]. In addition, *Schistosoma* spp. infection is typically thought to increase the Th2 immune response and lower the Th1 immune response, which causes increased levels of interleukines that may induce accelerated replication of HIV[6].

Both HIV testing and *Schistosoma* spp. testing are important to take into account when comparing studies looking at the interaction between both infections. The Tanzanian national guidelines for HIV testing follow WHO recommendations. The standard procedure for diagnosis of HIV in our study population involves the use of rapid tests for antibody testing[7] and since 2016, HIV RNA viral load quantification using PCR has been in place to evaluate response to ART[8]. Both CD4 counts and HIV RNA viral load change over the course of a natural HIV infection, and are predictors of the course of the HIV infection[9-11].

The current gold standard for diagnosis of active *Schistosoma* infection and detection of the species, as recommended by the WHO, is microscopy on urine or stool. It is less sensitive than other diagnostic strategies but has a near-perfect specificity and allows to differentiate between the infecting *Schistosoma* species if both urine and stool are provided. Schistosome antigen testing has been improved over the years to reach a high sensitivity and specificity for diagnosis of *Schistosoma* genus, although it does not allow distinction between *Schistosoma*

species[12]. Circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) can be detected either in the serum or in the urine of infected individuals, meaning the testing only requires one biological matrix to identify infection, and the level of these antigens is proportional to the intensity of infection[12]. The CAA and CCA based tests have been optimized for use in the field and CAA testing has been improved to be able to use dried blood spot samples for testing[13]. Current PCR techniques detect active infection but are expensive and often do not distinguish between *Schistosoma* species[12].

There is a clear association between *Schistosoma* spp. and HIV in women in the literature[14-18], and being infected with *Schistosoma* spp. increases the risk of HIV acquisition for women but not for men[19-22]. This is thought to be due to the difference in clinical urogenital schistosomiasis in men and women as mentioned above.

However there is still a lot unknown or poorly understood about HIV/*Schistosoma* spp. co-infections. Past studies have explored specific directions of the relationship between HIV and *Schistosoma* spp., looking separately on the one hand at the effect of *Schistosoma* spp. on HIV susceptibility and disease, and on the other hand at the effect of HIV on *Schistosoma* spp. infection. Most studies have looked at the effect of co-infections (in a cross-sectional way), while some have looked at the impact of *Schistosoma* spp. infection at time of HIV infection on HIV progression. There is still a lot of discrepancy in the literature regarding the role of *Schistosoma* spp. co-infection on HIV-RNA viral loads and CD4 counts, and regarding the role of HIV co-infection on *Schistosoma* spp. egg excretion. Based on schistosomiasis clinical differences between men and women, as well as differences in CD4 counts and HIV RNA viral load both between individuals and over the course of HIV infection, we would expect both sex and duration of HIV infection to be a main confounder in the relationship between HIV status and *Schistosoma* spp. infection.

The discussion will focus first on the conceptual framework presented in the introduction and the specific ways in which this thesis has strengthened our understanding of HIV/*Schistosoma* spp. co-infections. Then it will focus on the importance of taking into account HIV/*Schistosoma* spp. co-infections for prevention and diagnosis of both diseases, and finally it will indicate key limitations of most studies and recapitulate the questions that still remain to be answered.

II. REVISITING THE INITIAL CONCEPTUAL FRAMEWORK: WHAT GAPS DID WE FILL?

1) Effects of Schistosoma spp. infection on HIV susceptibility and disease

Studies had shown that co-infection with *Schistosoma* spp. increased HIV RNA viral load and viral load set point in humans[19, 23-25], as well as replication and reactivation of sHIV in macaques[26, 27]. As a result, it had been hypothesized for years that co-infection with *Schistosoma* spp. would hasten progression to HIV/AIDS outcomes and would increase transmission[28, 29]. Yet, to confirm these two assumptions, one needs longitudinal HIV/*Schistosoma* spp. co-infections studies. Ours were the first to try to answer these topics. The studies were built on the unique opportunity to have access to regular HIV testing results among a cohort of about 30,000 people over a period of 10 years and to be able to test the stored Dried Blood Spots (DBS) for the presence of *Schistosoma* spp. derived antigen indicating active infection at the time of DBS collection. The study results did not support the longstanding hypotheses but indicated the opposite (**Chapter 3 & 4**), explained in detail below.

A. <u>*Schistosoma* spp. infection at time of HIV infection slows down AIDS progression</u>

We found a highly significant protective effect of *Schistosoma* spp. infection against HIV outcomes (**Chapter 3**). This finding is unexpected if compared to studies showing increased HIV RNA viral loads during co-infection or shortly after HIV acquisition[19, 23-25]. However it is in line with our study results described in **Chapter 5**. **Chapter 5** indeed showed that *Schistosoma*-infected individuals have lower HIV RNA viral loads for a given duration of HIV infection as compared to HIV-infected individuals without *Schistosoma* infections, while lower viral loads are usually synonym of better HIV/AIDS outcomes[30]. It also coheres with the findings of multiple other studies that have reported increased CD4 counts and decreased HIV RNA viral loads in those with *Schistosoma* spp. infection, as compared to those without[31-35]. Therefore, we posit that host immune responses to *Schistosoma* spp. could be protective against HIV/AIDS progression, particularly after a longer duration of HIV infection, and that this can affect the host's clinical outcome.

One possible mechanism of this protective effect could be via induction of Th1 HIV-specific immunity in *Schistosoma* spp. co-infected individuals[36-39]. In addition, *Schistosoma* spp. infection leads to increases in Th17 and regulatory T (Treg) cells, which plays a critical role in determining the speed of HIV/AIDS progression[40, 41]. Lower Th17/Treg ratios have been associated with more advanced HIV infection[42], while absolute increases in Treg numbers have been associated with decreased markers of immune activation[42, 43], potentially leading to better HIV outcomes. In contrast, higher absolute numbers of Th17 cells could prevent microbial translocation and thereby decrease immune hyperactivation, which has been associated with poorer HIV outcomes[41, 42]. This, in combination with the previous immunological findings, suggests that there is not one way in which *Schistosoma* spp. infection influences HIV pathogenesis (and vice versa), but rather multiple pathways, that are

all balanced, and it is the ratios and balances and imbalances that will lead to an effect of one disease on the other[44-47]. The immunological pathways also likely change over time, making it essential to consider the length of infection with each disease and the duration of co-infection itself when trying to understand HIV and *Schistosoma* spp. co-infections.

B. *Schistosoma* spp. co-infection increases HIV transmission

While several studies have shown an increased risk of HIV acquisition of nearly 3 fold in women infected with *S. haematobium* or *S. mansoni*[19, 20], the risk of HIV transmission from men and women infected with *Schistosoma spp.* to healthy non-infected individuals is not as clear in our studies (**Chapter 4**). In our study population, *Schistosoma* spp. co-infection increased HIV transmission, although it was not statistically significant. The lack of significance was likely due to an overwhelming effect of the sex of the receiving partner on HIV transmission[48]. Women are indeed more susceptible to HIV infection than males[48-49]. To remove this effect of sex of the receiving partner, further studies should investigate the effect of *Schistosoma* spp. in HIV positive male individuals and the associated risk for transmission to female sero-discordant partners. In addition, it is possible that the effect was small in our study population because most people were infected with *S. mansoni*[13, 17, 18], thus having lower prevalence of genital schistosomiasis and/or a lower density of egg-induced local changes that may facilitate HIV transmission, compared to infection with *S. haematobium*. The fact that *Schistosoma* spp. seems to be associated with higher risk of HIV acquisition and transmission (**Chapter 4**), but associated with better HIV/AIDS outcomes and lower HIV RNA viral loads (**Chapter 3 & 5**), suggests a dual (and likely opposite) effect of *Schistosoma* spp.: a local effect, associated with genital schistosomiasis, and systemic immunological changes. We found that *S. mansoni* infection leads to increased HIV transmission but only with a small effect (**Chapter 4**). We believe that the effect of *S. haematobium* on HIV transmission would have been larger, due to higher frequency and severity of urogenital schistosomiasis, and reinforces the idea that the effect of *Schistosoma* spp. co-infection on HIV transmission could be due to local genital inflammation rather than systemic immunological changes[50-53].

C. *Schistosoma* spp. co-infection decreases HIV RNA viral load

Chapter 5 highlighted the need to account for the duration of HIV infection, through an approach taking into account CD4 counts at enrollment[54]. This is a straightforward calculation, requiring minimal data, to account for duration of HIV infection that we would suggest to include in future study on HIV and *Schistosoma* spp. co-infection. The technique behind this method has been used in multiple studies and is drawing from several data sources[54-59]. Time infected with HIV is defined as the sum of the time between HIV acquisition and enrollment at a clinic and the time between enrollment at a clinic and enrollment in the study. The time infected with HIV before enrolling in a clinic is defined using the first CD4 counts reported at the clinic, which is used to approximate the time delay between HIV infection and enrollment as a function of normal CD4 decay per calendar year

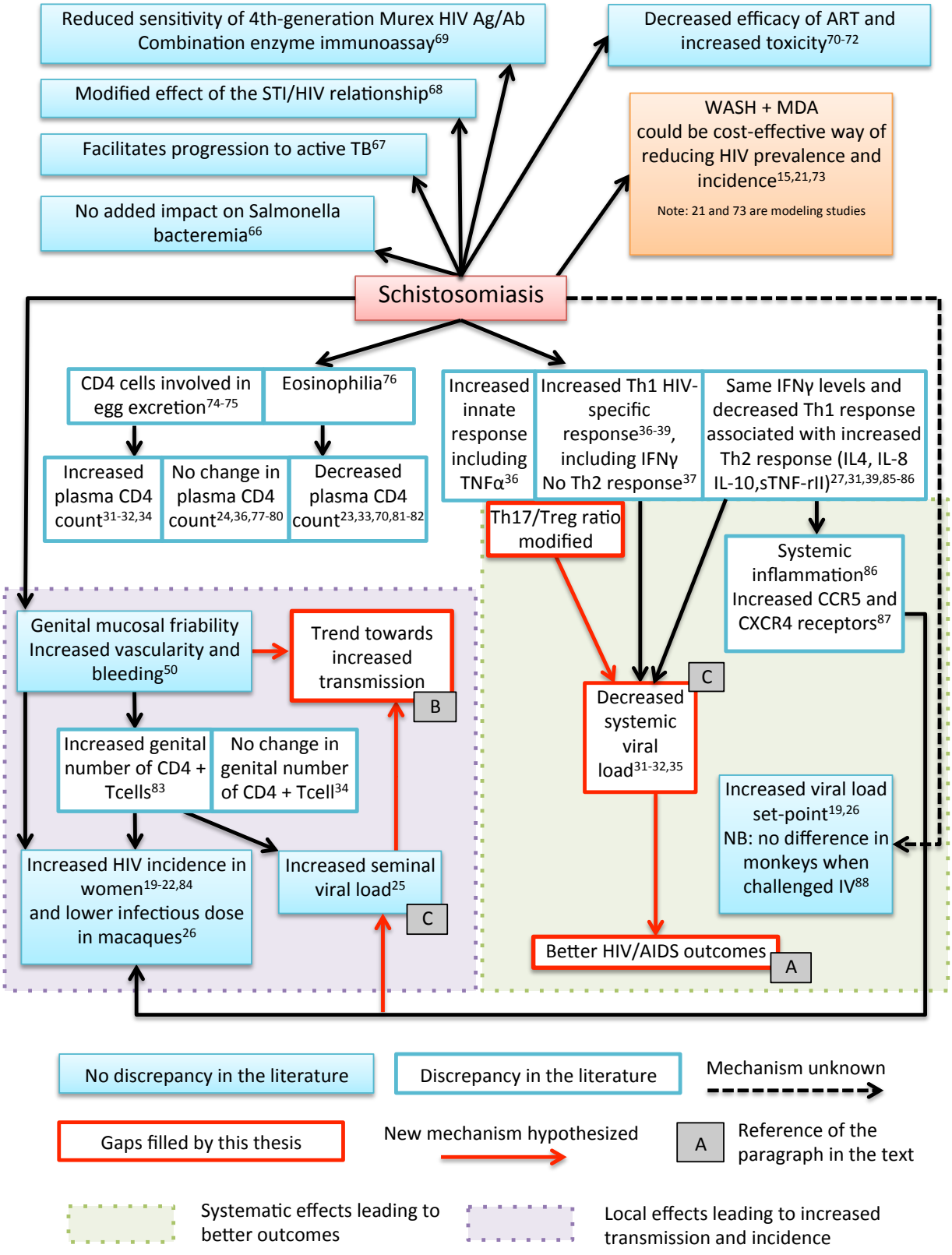
in drug naïve individuals. The time between the first CD4 count reported at the clinic and the date of study testing is then added to this variable to obtain the duration of HIV infection.

In our study, the results derived from this method concorded with results issued from surveillance data alone and approximation of the start of HIV infection by the middle date between two discordant DBS (**Chapter 2**). Several studies on HIV alone suggest that the relationship between immunological responses and HIV RNA viral load or CD4 count does not remain constant throughout the entire course of HIV infection, which further complicates the understanding of the immunological interactions between HIV infection and *Schistosoma* spp. infection[9-11, 60].

Duration of *Schistosoma* spp. infection is more difficult to measure than for HIV, as natural clearance and reinfection happen regularly in the adult population[61]. Even when people report having recently taken praziquantel (PZQ), yet test positive for *Schistosoma* spp., one cannot always distinguish between reinfection, decreased responsiveness to PZQ, and poor adherence[62-64]. As PZQ is made more widely available to populations in need, its administration and impact must be evaluated but the lack of gold standard diagnosis test for *Schistosoma* spp. further complicates analyses[65]. It can render studies that used microscopy and detected the presence of eggs in urine or stool difficult to compare to studies using, CAA or CCA and detecting worm derived antigens in blood or urine. Results are then also difficult to interpret. For example, how can a researcher measure the impact of HIV status on PZQ efficacy if he or she relies on microscopy since HIV status potentially impacts egg excretion?

The results from this thesis thus suggest two pathways through which *Schistosoma* spp. co-infections impact HIV epidemiology. The first pathway is a local one: genital mucosal friability and increased vascularity and bleeding, as well as increased seminal viral load lead to both increased transmission of HIV from an HIV/Schistosome co-infected individual to an HIV serodiscordant partner, and increased incidence of HIV in Schistosome infected women. The second pathway is a systemic one: Schistosomiasis leads to a modification in the Th17/Treg ratio, which in turn leads to decreased systemic viral load and better HIV/AIDS outcomes. We updated our conceptual framework of the mechanisms of the impact of *Schistosoma* spp. co-infections on HIV in **Figure 3.** The added mechanisms are shown in red.

Figure 1 - Updated conceptual framework of the epidemiology of HIV-Schistosome co-infections - Effect of Schistosoma spp. on HIV susceptibility and disease.



2) Effect of HIV on **Schistosoma** *spp. infection*

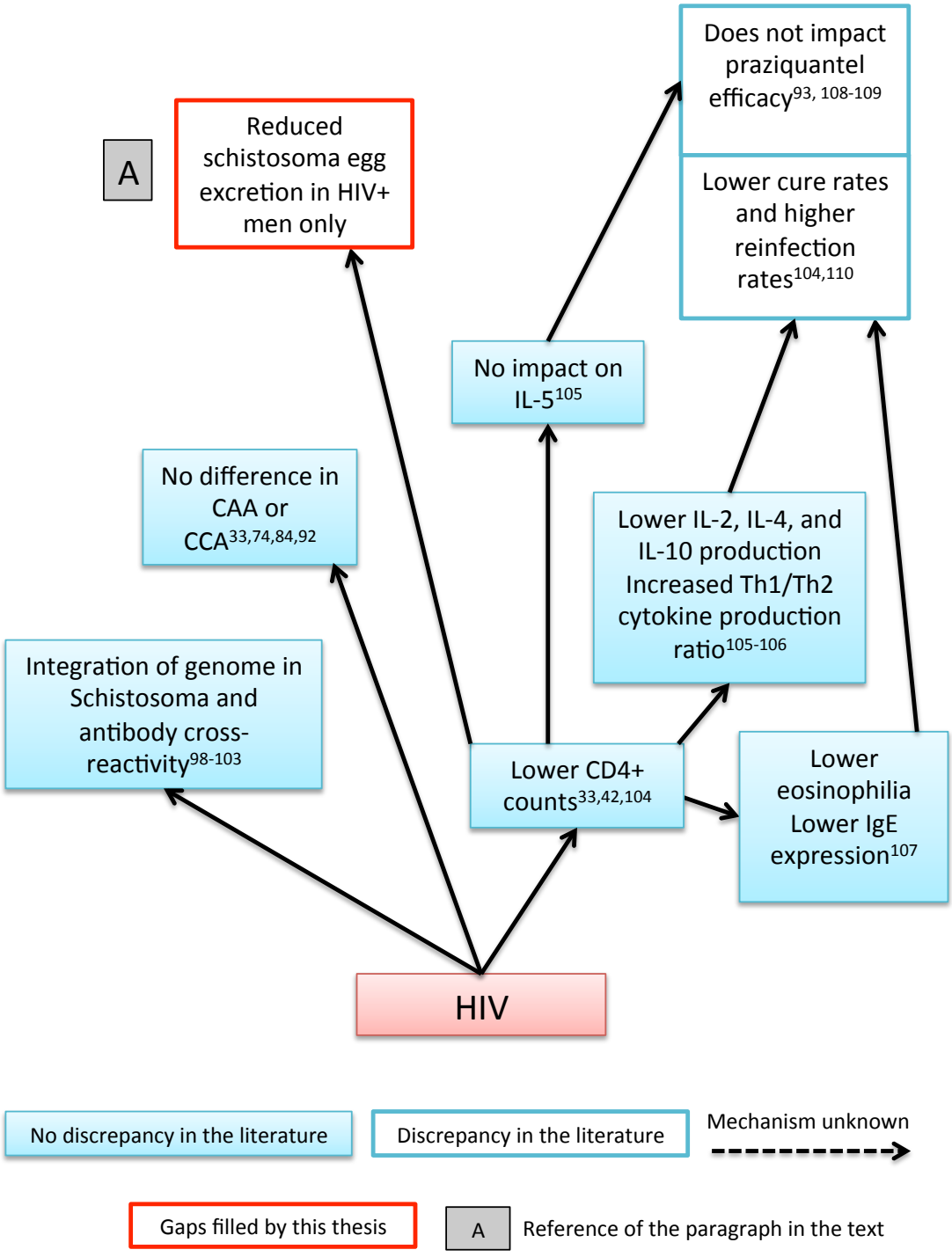
A. HIV co-infection reduces *Schistosoma* spp. egg excretion in men

Chapter 6 is the first large study to show an impact of HIV positivity on egg excretion. It demonstrates that the female to male ratio of the population studied was likely the main reason for conflicting results among previous studies[33, 74, 77, 89-93]. HIV infection status does not significantly affect *Schistosoma* spp. egg excretion in women, with the sensitivity of microscopy in both HIV-infected people and women only approximating 40%. Because most of the previous large studies done on this subject included mostly or entirely women, a difference in egg excretion based on HIV status was not detected[33, 77]. In contrast, the studies that included men, such as the Kenyan carwasher cohort, did report a difference in egg excretion[74]. The impact of sex on *Schistosoma* spp. infections is intriguging, with differential effects in women and men also demonstrated in epidemiologic studies of HIV acquisition[16, 17, 19, 94] and systemic immune responses to schistosome infection (Dupnik K, manuscript under review). Since the ratios of egg excretion to worm burden were lower in women infected with *S. mansoni*, the sex difference cannot be attributed to worm burden alone for this species. It is possible that CD4 counts could have been lower in men[95, 96] and that this could have impacted egg excretion via an effect on T cells[74, 75]. It is also possible that anatomical pelvic differences between men and women could lead to higher numbers of migrating parasite eggs trapped in female pelvic tissues than in males, or that worm fecundity could be affected by disparate immunological responses to *Schistosoma mansoni* worms in men versus women[53, 97].

The results of this thesis allowed us to explain part of the discrepancy in the results regarding the impact of HIV infection on *Schistosoma* pathophysiology: HIV infection leads to lower systemic CD4 counts, which in turn reduced *Schistosoma* spp. egg excretion in men but not in women. We updated our conceptual framework of the mechanisms of the impact of HIV co-infections on *Schistosoma spp.* in **Figure 4**. The added results are show in red.

Given the multiple studies indicating that host sex is a main distorting factor in the interaction between HIV and *Schistosoma* infections, sex should be taken into account and stratified for in any future study.

Figure 2 - Updated conceptual framework of the epidemiology of HIV-Schistosome co-infections - Effect of HIV on Schistosoma spp. infection.



III. IMPLICATIONS FOR DIAGNOSIS AND PREVENTION

1) Diagnosis of **Schistosoma** *spp.: microscopy testing is not recommended for women and HIV-infected individuals*

Chapter 6 highlighted the need for new WHO recommendations regarding the use of microscopy for detection of *Schistosoma* spp., especially in women and HIV-infected individuals. This is particularly important in the context of large reductions in the burden of schistosomiasis through mass drug administration[64]. As the prevalence of *Schistosoma* spp. infections decreases, the sensitivity of microscopy decreases too. One of the 2015-2030 United Nations Sustainable Development Goals is to eliminate schistosomiasis and other neglected tropical diseases altogether[111]. Given the ability of the diagnostic tool detecting the worm derived CAA that can now detect as little as one worm pair, and the CAA half-life of two days after successful killing of the worm[12, 112, 113], this test is extremely well-suited to quantify cure rates and to estimate the impact of HIV on response to PZQ treatment or reinfection.

CAA testing also has the advantage of reflecting the worm burden better than microscopy[12]. This allows detection of highly infected individuals, who might have differing immunity to *Schistosoma* compared to the rest of the population[114]. This in return allows for individual targeted treatment, and being able to detect highly infected individuals with unique immune responses might help better understand the immunological mechanisms at stake in *Schistosoma* spp. infections and co-infections.

In addition, Downs et al. came up with a proof-of-concept showing that DBS can be reliably used to quantify CAA: infection status can be determined even in DBS that had been stored for up to 8 years[13]. This opened new possibilities for other research studies on interactions between HIV and *Schistosoma* spp.. Of note, CAA testing is genus specific and additional testing is needed to indicate intestinal or urogenital infection[65, **Chapter 4**].

2) HIV prevention: targeting individuals present in **Schistosoma** *spp.-endemic areas to contain the HIV epidemic*

Our study results may add and improve current guidelines for HIV prevention. If *Schistosoma* spp. has a protective effect against poor HIV/AIDS outcome (**Chapter 3**), it does not mean that we should be stopping efforts to fight co-infections. Both diseases separately trigger millions of deaths and disabilities every year[1], and *Schistosoma* spp. infection is still a risk factor for HIV acquisition[19, 20], which is a main target of most HIV programs. Additional studies to determine whether PZQ treatment can prevent HIV acquisition should be prioritized, as PZQ treatment could be a safe, low-cost, acceptable way to prevent ongoing incident HIV infections in endemic regions. Current HIV/AIDS programs are aiming at improving care and ART availability for those who do get infected or were already infected in order to ultimately reduce the incidence of HIV[115]. **Chapter 2** shows that linkage to care is still poor in Tanzania and highlights the need for better integration from testing to enrollment into care. This chapter also calls for other ways to reduce the number of

people infected with HIV. Targeting individuals present in *Schistosoma* spp.-endemic areas, or those with known *Schistosoma* spp. infection, might help to curb the HIV epidemic. Both treating these individuals with PZQ and recommending more frequent HIV testing could lead to decreased seroconversion rates and better linkage to care. **Chapter 4** also suggests that future HIV/AIDS programs should focus their efforts on sero-discordant spouses.

Awareness regarding the relationship between HIV and *Schistosoma* spp. needs to be raised, not only among HIV/AIDS as well as *Schistosoma* spp. infection advocates, but clearly also among the general public. This could lead to an increase in the likelihood of HIV testing in populations exposed to both diseases such as fishermen, farmers, and those of lower socioeconomic status who do not have easy access to uncontaminated water sources. However, like with any disease campaign, public perceptions need to be managed appropriately and it will be essential to ensure that individuals with schistosomiasis do not become stigmatized[116], to keep high uptake of routine anti-schistosome treatment and HIV testing.

IV. REMAINING QUESTIONS AND METHOLOGY IMPROVEMENTS

1) More longitudinal studies are needed

At the epidemiological level, more longitudinal studies are needed to further clarify the impact of *Schistosoma* spp. infection on HIV RNA viral load set point, incidence, outcomes, and transmission. Longitudinal studies are often expensive, time-consuming, and ethically challenging[117]. A number of studies have used PZQ treatment to conclude on an effect of *Schistosoma* spp. infection on HIV pathogenesis[23, 25, 31, 35, 79, 86, 105]. Yet one cannot take the effect of PZQ treatment as a proxy for being *Schistosoma* spp.-free. In itself, PZQ treatment highly modifies the immune system response by killing the worms and thus activates a Th2 immune response[31]. The impact of PZQ treatment in co-infected individuals should be a whole research question(s) in itself, as should be the impact of ART treatment in co-infected individuals. It is challenging to compare a population of ART naïve HIV positive individuals in regards to HIV RNA viral load to a population not entirely ART naïve or on ART[118]. With the increasing use of DBS in HIV care for HIV RNA viral load testing[119], even in children, and the growing number of large sero-survey cohorts[120], CAA testing on DBS offers a cost-effective and convenient alternative to obtain human prospective data on the relationships between HIV and *Schistosoma* spp., with the potential to impact health policy and HIV prevention strategies throughout sub-Saharan Africa.

2) The immunological processes at stake are still not fully understood

At the immunological level, the impact of *Schistosoma* spp. infection on Th1 and Th2 immunity is still unclear in the context of HIV co-infections[27, 31, 36-39, 85, 86]. As mentioned earlier, the discrepancies are likely coming from an interpretation based on absolute immunological responses rather than ratios and imbalances, as well as the lack of a time variable in most immunological studies done until now.

In addition, the differences in the human immune response to *S. haematobium* and *S. mansoni* are still poorly understood[121]. While the local differences in the genital tract have been well studied[51-53], it is still unknown whether there are some systemic differences in the pathogenesis of the interaction between HIV and *S. haematobium* or HIV and *S. mansoni*. As the majority of our study population was infected with *S. mansoni* it is possible that we would have seen different effects, especially regarding transmission, in an *S. haematobium* infected population.

3) Children are missing from the picture

Finally, despite Bustinduy et al. raising the attention on the lack of studies on HIV/*Schistosoma* spp. co-infections in children in 2014[122], the literature on co-infections in children, or the impact of *Schistosoma* spp. infection during childhood on HIV infection as an adult, still only gathers one research study[123] and one literature review[122]. Given that the human immune response to schistosome infection changes with age and exposure, insight on co-infections in children will further add understanding to this complex issue[44].

V CONCLUDING REMARKS

The studies presented in this thesis have yielded further insight into the complex relationship between HIV and *Schistosoma* infection. They suggest a local interaction with urogenital schistosomiasis leading to increased incidence and transmission of HIV. They suggest as well a systemic interaction with *Schistosoma* spp. infection. The latter modifies immune responses to HIV, protecting against poor HIV outcomes. And HIV infection decreases CD4 counts, leading to decreased *Schistosoma* egg excretion.

The use of stored DBS from an HIV serosurvey cohort to test for *Schistosoma* spp. offered a unique opportunity to investigate the questions of outcomes and transmission in the context of HIV/*Schistosoma* spp. co-infections, by overcoming logistical and ethical difficulties associated with prospective cohort studies. Our work also highlighted the added benefit of using CAA as a diagnostic test for *Schistosoma* spp. Finally it offered new ways to include duration of HIV infection using CD4 counts in future research and suggested that *Schistosoma* spp. infection lowers HIV RNA viral load in ART naïve individuals.

Future research should focus on longitudinal studies and immunological studies, taking into account duration of infection, sex of the host, and infecting species of *Schistosoma*.

KEY FINDINGS

- People with *Schistosoma* spp. infection at the time of HIV-seroconversion develop adverse HIV outcomes more slowly than those without.
- Current infection with *Schistosoma* spp. is associated with significantly lower HIV viral loads after adjusting for time infected with HIV in ART naïve populations.
- Both women and HIV-infected individuals are significantly less likely to excrete *Schistosoma* spp. eggs when infected, even after controlling for a given worm antigen level.
- *Schistosoma* spp. infection in the transmitting partner has only a small effect on transmission of HIV due to the major effect of the sex of the receiving partner in HIV transmission
- Host sex and duration of HIV infection are likely main confounders, leading to the discrepancy in results in previous studies.
- More longitudinal studies are needed to further clarify the impact of *Schistosoma* spp. infection on HIV RNA viral load set point, incidence, outcomes, and transmission.
- Our Tanzanian study population demonstrates some moderate successes in linkage to care, with 80% of HIV-infected people knowing their HIV status and 62% of these being on ART.

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ADDENDUM:

ENGLISH SUMMARY
SAMENVATTING
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ENGLISH SUMMARY

Approximately 36.7 million people are infected with HIV around the world while 218 million are infected with *Schistosoma* spp.. These infections overlap and an estimated 6 million individuals are HIV/*Schistosoma* spp. co-infected. The majority of co-infections occur in Africa.

In the context of the HIV/AIDS epidemic, co-infections typically exacerbate morbidity and mortality. The immunodeficiency caused by chronic HIV infection increases the risk of co-infection with many pathogens. Moreover, administration of antiretroviral therapy (ART) does not always restore the pathogen-specific immune response to co-infections to normal levels. We might thus expect HIV infection to increase morbidity associated with endemicity of *Schistosoma* spp., and likely *vice versa*.

Several studies have showed a clear association between infection with *Schistosoma* spp. and HIV, and being infected with *Schistosoma* spp. increases a woman's risk of HIV acquisition. However, a lot is still unknown or poorly understood about HIV/*Schistosoma* spp. co-infections, both epidemiologically and immunologically. Longitudinal studies, controlling for sex, age, and duration of HIV infection are missing. The impact of *Schistosoma* spp. co-infection on HIV/AIDS outcomes is poorly described and the impact of *Schistosoma* spp. co-infection on HIV transmission is also not fully understood.
This thesis seeks to add to the current epidemiological knowledge on HIV and *Schistosoma* spp. co-infections, with the hope to understand the discrepancy in the data to date, and to generate new hypotheses and new questions for immunological studies. All studies presented in this thesis were conducted in the Lake Zone of Tanzania.

Chapter 1 reviews the literature to date when the work of this thesis started and the gaps in knowledge that this thesis tried to fill in.

Chapter 2 evaluated Tanzania's achievements regarding the 90-90-90 targets, which are a target for ART programs worldwide defined by the Joint United Nations Programme on HIV and AIDS (UNAIDS). It aims to achieve 90% of people living with HIV diagnosed (knowing their status), 90% of those diagnosed initiated on ART, and 90% of individuals on ART being virologically suppressed. Reaching the UNAIDS targets requires early diagnosis and effective linkage to and retention in care. Evaluating Tanzania's progress regarding HIV care allowed us to define our study population better, and to understand additional challenges in the fight against co-infections. Our Tanzanian study population demonstrated some moderate successes. The major gaps to optimizing linkage to care included a prolonged time from seroconversion to awareness of HIV status, as well as a low percentage of enrollment in care for ART treatment. Our results highlighted the importance of access and better integration of HIV services within the general healthcare system and to on-going serosurveys.

Chapter 3 indicated that people who had *Schistosoma* spp. infection at the time of HIV-seroconversion developed adverse HIV outcomes more slowly than those without *Schistosoma* spp. infection. Our study was unique in its use of banked dried blood spots to determine the impact of *Schistosoma* spp. infection on HIV disease progression, approximately 2-5 years after HIV-seroconversion. The findings suggested that the effect of co-infections on long-term outcomes might be milder than previously thought. They also highlighted an urgent need for longer-term clinical and immunological studies to confirm these outcomes.

Chapter 4 gave a first estimate of the hazard ratio of HIV-transmission from *Schistosoma* spp. co-infected transmitting partners compared to non-co-infected transmitting partners. It showed a trend towards an increased transmission of HIV, though suggested that the clinical impact may be small. Surprisingly, the *Schistosoma* spp. infection status of the receiving partner seemed not to be a risk factor for HIV acquisition. This may have occurred because sex of the receiving partner was so strongly associated with HIV seroconversion that any other risk factor for transmission became relatively inconsequential in our analysis. We were unable to investigate the effect of sex on the relationship between *Schistosoma* spp. and HIV transmission because 13 of 14 HIV-seroconversions occurred in women. This study also demonstrated a risk of HIV acquisition 19 times higher in serodiscordant couples than in the general population, highlighting the need for couples' targeted HIV counseling and testing as a strategic way to address the continuing incident HIV infections in Tanzania.

Chapter 5 signified that individuals with HIV and *Schistosoma* spp. co-infections have lower viral loads than those with HIV alone, when accounting for time infected with HIV. The difference in viral load was clinically significant and suggested a protective effect of *Schistosoma* spp. infection against long-term HIV outcomes and HIV transmission, which is in line with the results of **Chapter 3**. This study indicated that duration of HIV infection may be a critical explanatory factor in the disparate findings of studies on HIV viral load and *Schistosoma* spp. infections. Studies that examined viral loads earlier in the course of HIV infection may report increased viral load in the setting of *Schistosoma* spp. co-infections, and studies over the longer term may report lower viral loads. Studies that did not take into account duration of HIV infection also may have compared individuals at different stages of their HIV infection.

Finally, **Chapter 6** showed that both, women and HIV-infected individuals (males as well as females), were significantly less likely to excrete *Schistosoma* spp. eggs when hosting the parasite, even after controlling for a given worm antigen level. Our findings clarified reasons for divergent results of past studies on the relationship between HIV and egg excretion. This work suggested that current guidelines for the use of microscopy to diagnose *Schistosoma* spp. infections in HIV-infected individuals and in women merit reconsideration.

SAMENVATTING

Wereldwijd zijn naar schatting ongeveer 36,7 miljoen mensen besmet met HIV, terwijl 218 miljoen mensen geinfecteerd zijn met Schistosoma spp. Deze infecties overlappen elkaar en ongeveer 6 miljoen personen hebben een HIV/Schistosoma co-infectie. De meeste co-infecties komen voor in Afrika.

In de context van de HIV/AIDS-epidemie verergeren co-infecties doorgaans morbiditeit en mortaliteit. De immunodeficiëntie veroorzaakt door de chronische HIV-infectie verhoogt het risico op co-infectie met veel andere pathogenen. Ook wordt door het toedienen van antiretrovirale therapie (ART) niet altijd de pathogeen-specifieke immuunrespons tegen co-infecties tot normale niveaus hersteld. We zouden dus verwachten dat een HIV-infectie de morbiditeit van Schistosoma verhoogt en geassocieerd is met endemiciteit, en vice versa.

Verschillende onderzoeken hebben een duidelijk verband aangetoond tussen infectie met Schistosoma en HIV. Met name vrouwen met een Schistoma infectie hebben een verhoogd risico op het krijgen van HIV. Er is echter nog veel onbekend over HIV/Schistosoma co-infecties, zowel epidemiologisch als immunologisch. Longitudinale studies, die corrigeren voor geslacht, leeftijd en duur van HIV-infectie ontbreken. De impact van een Schistosoma co-infectie op HIV/AIDS ziekteprogressie is grotendeels onbekend evenals de impact van een Schistosoma co-infectie op HIV-transmissie.
Onderzoek in dit proefschrift is een aanvulling op de huidige epidemiologische kennis over HIV en Schistosoma co-infecties, om de discrepantie in de huidige data te overbruggen en nieuwe hypotheses voor immunologische studies aan te dragen. Alle studies die in dit proefschrift zijn beschreven zijn uitgevoerd in de Lake Zone van Tanzania.

Hoofdstuk 1 geeft een overzicht van de literatuur voor het schrijven van dit proefschrift en beschrijft de hiaten in kennis die de studies beschreven in dit proefschrift probeerde in te vullen.

In **hoofdstuk 2** evalueerden we de prestaties van Tanzania met betrekking tot de 90-90-90 doelen gedefinieerd door het 'Joint United Nations Program on HIV and AIDS' (UNAIDS) programma. Het doel is om 90% van de mensen met HIV gediagnosticeerd te hebben,90% van de gediagnosticeerde patienten te behandelen met ART, en dat in 90% van de mensen die behandeld worden met ART virologie gesupprimeerd is. Het bereiken van deze UNAIDS-doelen vereist een vroege diagnose van mensen die HIV-geïnfecteerd zijnen een effectieve koppeling aan zorg. Door de vooruitgang van Tanzania op het gebied van HIV-zorg te evalueren, konden we onze studiepopulatie beter definiëren en aanvullende uitdagingen beter begrijpen in de strijd tegen co-infecties. De belangrijkste struikelblokken in het optimaliseren van de koppeling aan de zorg zijn een langere tijd tussen seroconversie en bewustzijn van de HIV-status, en een laag percentage HIV-geïnfecteerde patiënten die zich inschrijven voor behandeling met ART. Onze resultaten benadrukken het belang van toegang tot en een betere integratie van HIV-diensten in de algemene gezondheidszorg en in lopende serosurveys.

In **hoofdstuk 3** zagen we dat mensen met een Schistosoma infectie ten tijde van HIV-seroconversie langzamer negatieve bijwerkingen ontwikkelden dan mensen zonder een Schistosoma infectie. Onze studie was uniek door het gebruik van in het verleden op filterpapier ingedroogde bloed samples ('banked dried blood spots') om de impact van een Schistosoma infectie op de progressie van HIV te bepalen 2-5 tot jaar na HIV-seroconversie. De bevindingen suggereerden dat het effect van co-infecties op de langetermijnresultaten mogelijk milder is dan eerder werd gedacht. Het benadrukte ook een dringende behoefte aan klinische en immunologische langere termijn studies op om deze resultaten te bevestigen.

Hoofdstuk 4 beschrijft een schatting van de risico-verhouding van HIV-transmissie van Schistosoma co-geïnfecteerde HIV-overdragende partners in vergelijking met niet-co-geïnfecteerde overdragende partners. Het toonde een trend richting een verhoogde kans op overdracht van HIV, hoewel de klinische impact waarschijnlijk klein zal zijn. Verrassend is dat de Schistosoma infectiestatus van de ontvangende partner geen risicofactor voor HIV-acquisitie leek te zijn. Dit kan zijn omdat het geslacht van de ontvangende partner zo sterk was geassocieerd met HIV-seroconversie dat elke andere risicofactor voor overdracht relatief onbelangrijk werd in onze analyse. We konden het effect van geslacht op de relatie tussen Schistosoma spp niet onderzoeken, omdat 13 van de 14 HIV-seroconversies plaatsvonden bij vrouwen. Deze studie toonde ook een risico aan van HIV-besmetting die 19 keer hoger was bij sero-afwijkende paren dan bij de algemene bevolking. Dit laat de noodzaak zien van doelgerichte HIV-counseling en -onderzoek voor paren als een strategische manier om de voortdurende incidentie van HIV-infecties in Tanzania aan te pakken.

Hoofdstuk 5 benadrukt dat personen met een HIV en Schistosoma co-infectie lagere virusconcentraties hebben dan personen die enkel een HIV-infectie hebben. Hierbij hielden wij rekening met de tijd sinds HIV-seroconversie. Het verschil in virale concentraties was klinisch significant en suggereerde een beschermend effect van Schistosoma infectie tegen langdurige HIV ziekteprogressie en transmissie. Dit is in overeenstemming met de resultaten van **hoofdstuk 3**. De duur van een HIV-infectie kan dus een essentiële verklarende factor kan zijn in de uiteenlopende bevindingen van studies naar virale concentraties bij HIV en Schistosoma infecties. Studies gericht op de virale concentraties in een vroeger stadium van de HIV-infectie , vinden waarschijnlijk verhoogde virale concentraties bij een co-infectie met Schistosoma co-infecties. Studies gericht op de langere termijn virale concentraties vinden mogelijk lagere virale concentraties vinden. Indien er geen rekening gehouden werd met de duur van een HIV-infectie, is het mogelijk dat er individuen vergeleken zijn die in een verschillend stadium van hun HIV-infectie verkeerden.

Ten slotte toonde **hoofdstuk 6** aan dat zowel vrouwen als HIV-geïnfecteerde personen (zowel mannen als vrouwen) aanzienlijk minder Schistosoma spp. eieren uit scheiden bij een infectie met Schistosoma spp zelfs wanneer er gecorrigeerd werd voor worm antigeen levels-. Onze bevindingen verduidelijken de redenen voor uiteenlopende resultaten van eerdere studies over de relatie tussen HIV en ei-excretie. Dit werk suggereerde dat de huidige richtlijnen voor het gebruik van microscopie om Schistosoma spp. infecties bij met HIV geïnfecteerde personen en vrouwen een heroverweging verdient.

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CURRICULUM VITAE

Soledad Colombe was born in Nevers, France, in 1991. In 2008 she entered a classe préparatoire which would prepare her for 2 years to the national entrance exam for French veterinary schools. She started studying veterinary medicine at the Veterinary School of Lyon, France in 2010. In parallel of her veterinary studies, she took classes in environmental health and biostatistics at the Université Lyon 1, France. She then started a Master in Public Health (MPH) at Yale University, USA, in 2014, in the epidemiology of infectious diseases' track, with a focus on global health. She graduated with both an MPH and a Doctorate in Veterinary Medicine in 2016. Upon graduation, she was hired by Weill Cornell University as a Global Health Fellow in Tanzania and conducted and coordinated studies on the epidemiology of HIV and *Schistosoma* spp. co-infections, with the collaboration of the Tanzanian National Institute for Medical Research and Leiden University Medical Center. These studies became the basis of her PhD. As a Global Health Fellow, she also taught microbiology of infectious diseases at the Catholic University of Health and Allied Sciences within Bugando Medical Centre, and mentored medical students and junior researchers.

Soledad is currently a fellow in the European Program for Intervention Epidemiology Training (EPIET) organized by the European Center for Disease Control (ECDC), and is based at the Public Health Agency of Sweden.

PEER-REVIEWED PUBLICATIONS

- Marti AI, **Colombe S**, Masikini PJ, Kalluvya SE, Smart LR, Wajanga BM, Jaka H, Peck RN, Downs JA. Increased hepatotoxicity among HIV-infected adults co-infected with Schistosoma mansoni in Tanzania: A cross-sectional study. PLoS Negl Trop Dis. 2017 Aug 17;11(8):e0005867. doi: 10.1371/journal.pntd.0005867. eCollection 2017 Aug.
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