Evaluating risk factors and synergistic effects of two common HIV-1 coinfections: schistosomiasis and trichomoniasis

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Abstract

Improving our understanding of two common HIV-1 coinfections: schistosomiasis and trichomoniasis

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Introduction: HIV-1 coinfections have long been suspected of catalyzing the HIV-1 epidemic by increasing HIV-1 transmission or acquisition risk. The geographical distribution of coinfections may explain why some regions have been more heavily impacted by HIV-1. The primary objective of this dissertation was to expand our understanding of two common HIV-1 coinfections that have been hypothesized to increase the risk of HIV-1 transmission and acquisition: schistosomiasis and trichomoniasis. The specific aims of this dissertation were 1) Identify correlates of *T. vaginalis* infection within a population of HIV-1 serodiscordant heterosexual couples, 2) Estimate the association between schistosomiasis and HIV-1 acquisition, 3) Evaluate the impact of schistosome coinfection on HIV-1 set-point genital viral load levels, and 4) Evaluate the association between schistosome coinfection and HIV-1 set-point plasma viral load levels.

Methods: To conduct these analyses, we used data from four cohort studies: the Partners in Prevention HSV/HIV Transmission Study, the Couples Observational Study, the Partners PrEP Study, and the Mombasa Cohort. All analyses utilized data from multiple cohorts. For all cohorts, a large amount of individual-level information was collected, including characteristics associated with HIV-1 acquisition risk and the prevalence of coinfections, permitting thorough adjustment for possible confounding factors.

Results and Conclusions: Correlates of *T. vaginalis* infection: In a cross-sectional analysis of 8,155 HIV-1 serodiscordant couples, the strongest predictor of a prevalent *T. vaginalis* infection was having an infected sexual partner. Thus, concurrent treatment of sexual partners is critical to prevent reinfection. Among women, having a circumcised male partner was associated with reduced *T. vaginalis* risk while bacterial vaginosis (detected via Nugent Score) was associated with an increased risk, so expanding male circumcision programs and bacterial vaginosis treatment has the potential to reduce the prevalence of trichomoniasis. Schistosomiasis and HIV-1 acquisition risk: In nested case-control analyses including 575 HIV-1 seroconverters and 1,675 controls, *S. mansoni* infection was not associated with an increased the risk of HIV-1 acquisition. In addition, S*. haematobium* infection was not associated with a statistically significant increase in HIV-1 acquisition risk, though our result suggested that women with S*. haematobium* could face a moderate increased risk of HIV-1 acquisition. Schistosomiasis and set point HIV-1 RNA viral loads: Schistosomiasis was not associated with increased plasma HIV-1 viral loads. Our results do not support the hypothesis that schistosome coinfection increases the rate of HIV-1 disease progression. Schistosomiasis and genital HIV-1 RNA viral loads: Schistosomiasis was not associated with increased genital HIV-1 viral loads. Our results do not support the hypothesis that schistosome coinfection increases HIV-1 transmission risk.

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Acknowledgments

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Chapter 1. Introduction

This dissertation expands our understanding of two HIV-1 coinfections that are common in sub-Saharan Africa: trichomoniasis and schistosomiasis. Trichomoniasis, caused by the protozoan parasite *Trichomonas vaginalis*, is the most prevalent curable sexually transmitted infection globally, with an estimated 187 million infected individuals aged 15 to 49 years old [1]. Schistosomiasis, a parasitic disease caused by the schistosome flatworm, affects approximately 200 million people worldwide [2].

Though both diseases can be easily treated, public health programs in sub-Saharan Africa have not succeeded in substantially reducing the prevalence of either disease. Trichomoniasis can be treated with a 1- or 7-day course of metronidazole [3]. However, identifying *T. vaginalis* infections is challenging because they are usually asymptomatic [4]. Previously, *T. vaginalis* screening has relied on wet mount or culture diagnostic assays, which are time-consuming and lack sensitivity. Schistosomiasis is treated with a single dose of praziquantel at an estimated cost of US $0.20 per treatment [5]. Though the World Health Organization recommends preventive chemotherapy for schistosomiasis to reduce morbidity, funding for such programs has been limited, and treatment programs are not currently implemented in many endemic regions [5, 6].

Other HIV-1 coinfections, such as herpes simplex virus type 2 (HSV-2) and malaria, play an important role in the HIV-1 epidemic by heightening susceptibility to HIV-1 infection and increasing the infectiousness of those with HIV-1 [7-9]. For example, in longitudinal cohort studies HSV-2 increases the risk of HIV-1 acquisition by 2- to 3-fold [7], while increased viral load associated with malaria coinfection has been estimated to account for a sixth of HIV-1 transmission in Kenya [10].

Findings suggest that HIV-1 coinfection with trichomoniasis or schistosomiasis may similarly increase the risk of HIV-1 acquisition and HIV-1 transmission. Observational data suggest that trichomoniasis may increase women's risk of HIV-1 acquisition by 1.5- to 3-fold [11]. For both men and women with HIV-1,

trichomoniasis coinfection has been associated with increased genital shedding of HIV-1 [11], suggesting that trichomoniasis increases HIV-1 transmission risk [8].

For schistosomiasis, four cross-sectional studies, limited by small sample sizes and minimal adjustment for possible confounders, suggested that schistosome infection increases the risk of HIV-1 acquisition by 2- to 6-fold [12-15], findings which have received considerable attention [16]. Based on these results, mathematical modeling estimates that annual mass treatment of school-age children could reduce the prevalence of HIV-1 in Kenya by 16% [17]. A recent study found higher set point viral load levels among individuals with schistosomiasis at the time of HIV-1 seroconversion [18], implicating a role for schistosomiasis increasing HIV-1 disease progression. This findings is supported by studies showing that schistosome-infected macaques had higher viral loads after SHIV acquisition compared to schistosome-uninfected controls [19, 20]. Biological mechanisms by which schistosomiasis could heighten HIV-1 risk include recruitment of HIV-1 target cells [21], breaches in the cervical mucosa caused by schistosome eggs [22], and chronic inflammation, mechanisms similar to those by which other coinfections have been found to heighten HIV-1 susceptibility and infectiousness [9].

Analyses conducted within this dissertation capitalized on substantial prior investments by conducting secondary analyses and sample testing using biological samples and data from large, high-quality prospective studies in two key at-risk populations from Kenya and Uganda – HIV-1 serodiscordant couples and female sex workers. To better understand the epidemiology of trichomoniasis within couples, we conducted a large cross-sectional analysis utilizing data from both members of sexual partnerships. For schistosomiasis, we tested stored serum samples using a laboratory algorithm including antibody and antigen testing to identify both active and resolved infections, while also using antigen concentrations as a measure of infection intensity. Immunoblots identified the species of schistosome responsible for the infection, permitting sub-analyses by schistosome species. We then prospectively evaluated if schistosome infection was associated with several outcomes: HIV-1 acquisition, genital set point viral load levels, and plasma set point viral load levels. These datasets have been previously interrogated for studies of other novel factors predicting HIV-1 acquisition, transmission, and disease progression, and key potential

confounders were well-measured for these populations, allowing for thorough confounders adjustment [8, 23-25].

Chapter 2: A cross-sectional analysis of *Trichomonas vaginalis* infection among heterosexual HIV-1 serodiscordant African couples

Aim 1: **Identify correlates of TV infection within heterosexual couples**

Approach: We conducted a large cross-sectional analysis among African HIV-1 serodiscordant heterosexual couples. We assessed how characteristics of both members of sexual partnerships were associated with the prevalence of male and female trichomoniasis infection.

Hypothesis*:* There are modifiable risk factors for trichomoniasis, which have the potential to be addressed by public health interventions.

Chapter 3: Associations between schistosomiasis and HIV-1 acquisition risk in four prospective cohorts

Aim 2: **Estimate the association between schistosomiasis and HIV-1 acquisition risk**

Approach*:* We conducted a nested case-control analysis to test whether schistosome infection was associated with HIV-1 acquisition during study follow-up.

Hypothesis*:* Schistosome infection is associated with an increased risk of HIV-1 acquisition.

Chapter 4: Effects of schistosomiasis on HIV-1 plasma and genital set point viral loads

Aim 3: **Estimate the impact of schistosomiasis on HIV-1 genital viral load in HIV-1 infected women – a marker of HIV-1 transmission risk**

Approach*:* We measured HIV-1 viral shedding in endocervical and vaginal swabs collected from women who participated in a cohort of female sex workers. We tested whether schistosomiasis was associated with levels of HIV-1 viral shedding in genital samples, which is an established market of HIV-1 infectiousness.

Hypothesis*:* Schistosome coinfection is associated with increased levels of HIV-1 RNA in the genital tract. This association may be stronger among individuals infected by *S. haematobium* than those infected by *S. mansoni.*

Aim 4: **Evaluate the association between schistosome coinfection and HIV-1 plasma set-point viral load – a marker for the rate of HIV-1 disease progression**

Approach*:* Among HIV-1 seroconverters, we tested whether schistosomiasis was associated with higher plasma set point HIV-1 viral load, measured 4-24 months after acquisition of HIV-1.

Hypothesis: Schistosome coinfection is associated with higher HIV-1 RNA set-point in plasma.

Chapter 2. A cross-sectional analysis of *Trichomonas vaginalis* infection among heterosexual HIV-1 serodiscordant African couples

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A cross-sectional analysis of *Trichomonas vaginalis* infection among heterosexual HIV-1 serodiscordant African couples

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ABSTRACT

Objectives *Trichomonas vaginalis* (TV) is the most prevalent curable sexually transmitted infection worldwide and has been associated with adverse health outcomes and increased HIV-1 transmission risk. We conducted a cross-sectional analysis among couples to assess how characteristics of both individuals in sexual partnerships are associated with the prevalence of male and female TV infection.

Methods African HIV-1 serodiscordant heterosexual couples were concurrently tested for trichomoniasis at enrolment into two clinical trials. TV testing was by nucleic acid amplification or culture methods. Using Poisson regression with robust standard errors, we identified characteristics associated with trichomoniasis.

Results Among 7531 couples tested for trichomoniasis, 981 (13%) couples contained at least one infected partner. The prevalence was 11% (n=857) among women and 4% (n=319) among men, and most infected individuals did not experience signs or symptoms of TV. Exploring concordance of TV status within sexual partnerships, we observed that 61% (195/319) of TV-positive men and 23% (195/857) of TV-positive women had a concurrently infected partner. In multivariable analysis, having a TV-positive partner was the strongest predictor of infection for women (RR 4.70, 95% CI: 4.10-5.38) and men (RR 10.09, 95% CI: 7.92-12.85). For women, having outside sex partners, gonorrhoea, and intermediate or high Nugent scores for bacterial vaginosis were associated with increased risk of trichomoniasis, whereas age 45 years and above, being married, having children, and injectable contraceptive use were associated with reduced trichomoniasis risk. Additionally, women whose male partners were circumcised, had more education, or earned income had lower risk of trichomoniasis.

Conclusions We found that within African HIV-1 serodiscordant heterosexual couples, the prevalence of trichomoniasis was high among partners of TV-infected individuals, suggesting that partner services could play an important role identifying additional cases and preventing reinfection. Our results also suggest that male circumcision may reduce the risk of male-to-female TV transmission.

[abstract 300 words]

INTRODUCTION

The protozoan parasite *Trichomonas vaginalis* (TV) is the most prevalent curable sexually transmitted infection worldwide, with an estimated 187 million infected individuals aged 15 to 49 years old [1]. Trichomoniasis has been associated with adverse outcomes in women, including pelvic inflammatory disease, low birth weight, and preterm delivery [26, 27]. In addition, observational data suggest that TV may increase women's risk of HIV-1 acquisition by 1.5-3 fold [11]. For both men and women with HIV-1, TV coinfection has been associated with increased genital shedding of HIV-1 [11], suggesting that TV increases HIV-1 transmission risk [8].

Trichomoniasis symptoms for women can include vaginal discharge, dysuria, itching, vulvar irritation, and abdominal pain while men may experience urethritis [28]. Though trichomoniasis can be easily treated with a 1- or 7-day course of metronidazole [3], identifying TV cases is challenging because the infection is usually asymptomatic [4]. Previously, TV screening has relied on wet mount or culture diagnostic assays, which are time-consuming and lack sensitivity. Recently developed nucleic acid amplification tests (NAAT) have improved diagnostic sensitivity, especially among men [29]. However, the cost and infrastructure requirements for NAAT testing currently prohibits widespread clinical use, and affordable point-of-care TV diagnostics are needed to support widespread testing, diagnosis, and treatment.

The prevalence of trichomoniasis worldwide is higher among women than men [1]. A cause of this disparity is that the average duration of untreated infection is longer for women than men. Though data are limited, the average duration of untreated infection was estimated to be 18 months for women compared to 1.5 months for men [30]. The high prevalence and relatively short duration of infection suggests that for TV to be sustained in populations, individuals (especially men) are likely to be repeatedly infected by sexual partners. Although ongoing sexual partnerships are a central component of TV epidemiology, few recent studies have explored the prevalence and correlates of trichomoniasis within couples [31-33].

Since widespread screening for trichomoniasis is resource-intensive and not currently practical in most settings, identifying and targeting interventions towards modifiable risk factors for trichomoniasis may present the best approach to reduce the burden of TV and TV-associated morbidity. To identify correlates

of TV infection, we conducted a large cross-sectional analysis among African HIV-1 serodiscordant heterosexual couples. We assessed how characteristics of both members of sexual partnerships were associated with the prevalence of male and female TV infection.

METHODS

Study population

Data from two prospective cohorts of African heterosexual HIV-1 serodiscordant couples were included in this analysis. The Partners in Prevention HSV/HIV Transmission Study was a randomized, placebo-controlled trial of daily acyclovir as HSV-2 suppressive therapy to reduce HIV-1 transmission from HSV-2/HIV-1 coinfected individuals to their HIV-1 uninfected partners. Between November 2004 and April 2007 the study enrolled 3408 couples from 14 sites in seven African countries [34]. The Partners PrEP Study was a randomized, placebo-controlled trial of pre-exposure prophylaxis (PrEP) to prevent HIV-1 acquisition. Serodiscordant couples were randomized to daily tenofovir disoproxil fumarate, combination tenofovir disoproxil fumarate and emtricitabine, or placebo. Between July 2008 and November 2010, 4747 heterosexual HIV-1 serodiscordant couples were enrolled at nine sites in Kenya and Uganda [35].

Enrolment and exclusion criteria were similar in both parent studies: Participants were aged 18 and above, and eligible couples reported regular sexual intercourse with their study partner in the three months prior to enrolment and intention to remain together for the duration of the study. Exclusion criteria at enrolment included that the HIV-1 infected partner could not be on antiretroviral therapy or be eligible for initiation of antiretroviral therapy according to national guidelines, and women were excluded if they were pregnant. For the Partners in Prevention HSV/HIV Transmission Study, all HIV-1 infected partners were HSV-2 seropositive. There were no exclusions for individuals with syndromic or etiological STI diagnoses.

At the enrolment visit, interviewer-administered standardized questionnaires were used to collect information on demographics and sexual behaviours from both study partners. Genital exams were performed to identify STI symptoms. Clinician-collected endocervical swabs and urine samples were gathered from women and men respectively to test for trichomoniasis, chlamydia, and gonorrhoea while blood samples were collected for serologic assays.

Both study protocols, including planned analyses for STI transmission risk factors, were approved by the University of Washington Human Subjects Review Committee (IRB ID: STUDY00000172 and STUDY00000867) as well as ethics review committees at each study site. All study participants provided written informed consent.

Laboratory methods

All samples used for diagnostic testing were collected at the study enrolment visit, which both members of the couple attended together. Testing for TV was done using NAAT by APTIMA TV TMA (seven sites in the Partners PrEP Study; Gen-Probe, San Diego, CA), a research version of the APTIMA assay (all sites in the Partners in Prevention HSV/HIV Transmission Study), or by culture using InPouch TV (two sites in the Partners PrEP Study; Biomed Diagnostics, White City, OR).

HIV-1 serostatus was determined by rapid testing, with positive results confirmed by Western blot or ELISA. *N. gonorrhoeae* and *C. trachomatis* testing was performed using APTIMA Combo 2 (Gen-Probe, San Diego, CA) or COBAS Amplicor (Roche Diagnostics, Indianapolis, IN). Serologic testing for syphilis was by rapid plasma reagin testing, confirmed with the use of a treponema-specific assay. Gram-stained smears were prepared from vaginal swabs to evaluate vaginal microbiota. Normal microbiota, intermediate microbiota, and bacterial vaginosis were defined by Nugent scores of 0-3, 4-6, and 7-10, respectively.

Statistical methods

All couples in which both partners provided a sample for TV testing at study enrolment were included in this analysis. The Wilcoxon matched-pairs signed-rank test was used to assess if the prevalence of TV differed in men and women. Poisson regression models with robust standard errors were used identify factors associated with the risk of a prevalent TV infection. The association between genital symptoms and TV infection were assessed in bivariate models and in models adjusting for the presence of other STIs. Correlates of a prevalent TV infection for men and women were also analysed using Poisson regression. Factors associated with TV infection in bivariate models ($p<0.05$) were included in multivariable models. For factors with more than two categories, likelihood ratio tests were used to determine inclusion in

multivariable models. Analyses were performed using Stata version 13.1 (Stata Corporation, College Station, TX).

RESULTS

Of the 8155 HIV-1 serodiscordant couples enrolled in the Partners in Prevention HSV/HIV Transmission and Partners PrEP Studies, 92% (7531/8155) of couples were tested for trichomoniasis at enrolment. This included 4872 couples (65%) with a HIV-1 seropositive female partner and 2659 couples (35%) with a HIV-1 seropositive male partner (Table 1). The median age was 30 years for women and 36 years for men. Most couples were married, living together, and had been together for more than five years. Unprotected sex in the prior month was reported by 33% of couples, while 0.9% of women and 10% of men reported sex with individuals besides their primary partner in the prior month.

TV infection was detected in 1176 (7.8%) study participants, with the prevalence of infection 2.7-times higher among women than men (4.2% vs. 11.4%, $P < 0.001$). At least one partner tested positive for trichomoniasis in 13% (981/7531) of couples: 8.8% (662/7531) of couples had only the female partner positive, 1.6% (124/7531) of couples had only the male partner positive, and 2.6% (195/7531) of couples had both partners positive. Exploring concordance of TV status within sexual partnerships, we observed that 61% (195/319) of men with trichomoniasis had an infected female partner and 23% (195/857) of women with trichomoniasis had an infected male partner.

Women with abnormal vaginal discharge (RR 1.58, 95% CI: 1.35-1.84), cervical bleeding (RR 1.65, 95% CI: 1.41-1.92), or adnexal, cervical, or uterine tenderness (RR 1.42, 95% CI: 1.04-1.95) at their clinical exam were more likely to have trichomoniasis than women without those respective signs and symptoms (Table 2). These associations remained statistically significant after adjusting for other sexually transmitted infections. However, the majority (65%, 555/852) of women with trichomoniasis had none of these three signs or symptoms present, with 20% (173/852) of infected women experiencing abnormal vaginal discharge, 20% (169/852) of infected women experiencing cervical bleeding, and 4% (34/852) of infected women experiencing adnexal, cervical, or uterine tenderness. Among men, urethral discharge was uncommon.

Several factors were associated with the risk of a woman having a prevalent TV infection (Table 3). Multivariable analysis found that women with outside sex partners (RR 1.78, 95% CI: 1.15-2.76), HIV-1 (RR 1.19, 95% CI: 1.02-1.38), *N. gonorrhoeae* (RR 1.65, 95% CI: 1.23-2.23), and intermediate (RR 2.04, 95% CI: 1.70-2.44) or high (RR 1.96, 95% CI: 1.67-2.30) Nugent scores for bacterial vaginosis had increased risk of TV infection. Being married (RR 0.74, 95% CI: 0.59-0.93), having children (RR 0.80, 95% CI: 0.69-0.92), and using injectable contraception (RR 0.77, 95% CI: 0.64-0.94) were associated with reduced TV risk. In addition, women aged 45 and above were found to have a lower risk of TV (RR 0.63, 95% CI: 0.44-0.89) compared to women aged 18-24. Women whose male partner was circumcised (RR 0.82, 95% CI: 0.72-0.94), had nine or more years of education (RR 0.72, 95% CI: 0.63-0.82), or who earned income (RR 0.82, 95% CI: 0.71-0.94) were less likely to have trichomoniasis.

For men, concurrent HIV-1 infection (RR 0.69, 95% CI: 0.53-0.90) was associated with decreased risk of TV infection (Table 4). Men whose samples were tested by NAAT had increased risk of being identified as having trichomoniasis (RR 2.43, 95% CI: 1.27-4.65) compared to men tested by culture.

For both women and men, having a partner with trichomoniasis was the factor most strongly associated with TV infection risk. In unadjusted analyses, women with a TV-positive male partner had 6.66-times the prevalence (95% CI: 5.94-7.46) of TV infection, while men with a TV-positive female partner had 12.25-times the prevalence (95% CI: 9.89-15.16) of TV infection compared to individuals whose primary partner was TV-negative. The corresponding absolute change in the prevalence of TV associated with having a TV-positive partner was also large, with the prevalence increasing from 9% among women whose partner was TV-negative to 61% among women whose partner was TV-positive (risk difference = 52%). For men, the prevalence of TV increased from 2% among men whose partner was TV-negative to 23% among men whose partner was TV-positive (risk difference = 21%). In multivariable analyses, women with a TV-positive male partner had 4.70-times the prevalence (95% CI: 4.10-5.38) of TV infection, while men with a TV-positive female partner had 10.09-times the prevalence (95% CI: 7.92-12.85) of TV infection compared to individuals whose primary partner was TV-negative.

DISCUSSION

Using data from two large trials that enrolled HIV-1 serodiscordant couples living in sub-Saharan Africa, this exploratory analysis identified characteristics of both individuals in heterosexual couples that were associated with trichomoniasis. For both men and women, having a TV-infected partner was the factor most strongly associated with the likelihood of a prevalent TV infection. Our finding that 23% of women and 61% of men with TV had a partner with a concurrent TV infection highlights the importance of treating sexual partners of individuals diagnosed with TV for achieving good clinical outcomes. This will be increasingly important as the use of NAAT testing for TV expands and more male TV cases are identified, since the prevalence of trichomoniasis was approximately 3-fold higher among partners of TV-positive men compared to partners of TV-positive women. We also found that the prevalence of trichomoniasis among women was 2.7-times higher than among men, which is strikingly different from the existing WHO African Region estimates that women are 10-times more likely than men to have trichomoniasis [1]. WHO had few data points available to estimate of the prevalence of TV among men and used a complex methodology that attempted to adjust for the sensitivity and specificity of specific diagnostic tests to generate their estimates [30], which our findings suggest may not accurately reflect the distribution of trichomoniasis among women and men.

This study found that women with gonorrhoea, HIV-1, and intermediate or high Nugent scores for bacterial vaginosis were more likely to have trichomoniasis. The association with bacterial vaginosis has the potential to be especially important from a public health perspective because a large proportion of women in some populations have intermediate or high Nugent scores (48% of this study population). Cohort studies have found that women with intermediate or high Nugent scores were more likely to acquire TV [29, 36-39], and our analysis confirms this association while adjusting for characteristics of both members of sexual partnerships. In addition, the unadjusted analysis found that men whose female partners had intermediate or high Nugent scores had over a 2-fold increased risk of TV infection. Though this association was no longer significant in the multivariable model which adjusted for female partner TV status, the expected mediator of the association, this finding highlights the potential impact of bacterial vaginosis on the prevalence of TV among men.

Additionally, this analysis found that injectable contraceptive use was associated with a decreased risk of trichomoniasis. Several recent well-powered studies have also found injectable contraceptive use associated with decreased risk of TV infection [36, 37, 40-42]. It has been hypothesized that injectable contraceptive use reduces TV risk though inhibition of exogenous oestrogen and androgen receptors or by reducing iron availability through decreased menstrual flow [42, 43]. Additional research is needed to better understand the mechanism underlying this association.

This study confirms the results of a randomized controlled trial which observed that women with circumcised male partners were less likely to have trichomoniasis [44]. Two longitudinal studies that assessed the association between female TV acquisition and male partner circumcision status found mixed results, though these studies relied on female partners to report the circumcision status of their male partner and used lower-sensitivity wet mount assays to detect TV infection [45, 46]. We did not find an association between male circumcision and the risk of trichomoniasis among men, though data from other studies suggest a modest protective effect [47-50]. The mechanism through which male circumcision reduces the risk of male-to-female TV transmission is currently unknown, though it has been hypothesized that moisture in the subpreputial space in uncircumcised men may facilitate TV survival, increasing female exposure to the pathogen [44].

We identified few factors associated with trichomoniasis risk among men. Our data suggest that a male's risk of TV was largely driven by having a TV-positive female partner, consistent with evidence that the average duration of infection among men is short and thus recent exposure to TV is a prerequisite for infection. It is worth noting that though we found a protective effect of HIV-1 on men's risk of TV in our multivariable model, the study population consisted of HIV-1 serodiscordant couples so all HIV-1 negative men had HIV-1 positive female partners.

Limitations of this analysis should be considered when interpreting these results. Since the analysis was cross-sectional, we are unable to assess temporality and cannot determine if factors are associated with incident TV infections. Though NAATs were used for the majority of TV testing, 16% of couples were tested using culture. Culture is less sensitive at detecting TV infections, especially among men, so we may have underestimated the prevalence of TV [4, 51]. Including only those individuals tested by NAAT, the

prevalence of TV among women rose from 11% to 12% (770/6339) and among men rose from 4% to 5% (303/6339). Lastly, these cohorts of HIV-1 serodiscordant couples in stable partnerships are unique, and some results such as the prevalence estimates may not be generalizable to other populations.

Strengths of this analysis include the fact that we explored the epidemiology of TV within sexual partnerships. Adjusting for characteristics of the male partner, the female partner, and the couple allowed us to thoroughly explore factors associated with TV risk. The fact that we interviewed and performed genital exams for both members of sexual partnerships, rather than relying on one individual to report their partner's characteristics, likely reduced misclassification [52]. Our ability to analyse data from 7531 couples provided us with the statistical power to identify associations between TV and factors that have weaker associations or are less common. In addition, much of the existing data on TV concordance within couples comes from sexually transmitted disease clinics, where patients are likely to be experiencing symptoms. Most TV cases are asymptomatic [4], so our concordance data may better reflect the general population of TV cases.

In summary, trichomoniasis was more prevalent among women than men and was common in partners of TV-infected individuals, while most infected individuals did not have signs or symptoms of infection. These findings illustrate the importance of notifying the sexual partners of individuals diagnosed with trichomoniasis to identify additional cases and prevent reinfection. Our findings that female partners of circumcised men had an 18% reduced risk of prevalent trichomoniasis compared to women with uncircumcised partners suggests that male circumcision may be a useful public health intervention to prevent TV infections.

Key messages

- For both men and women, the strongest predictor of a prevalent TV infection is having a TV-infected sexual partner.
- Concurrent treatment of sexual partners is critical to prevent reinfection.

- Male circumcision programmes and expanded treatment for bacterial vaginosis have the potential to reduce the prevalence of trichomoniasis.

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Table 2.1 Characteristics of study participants

	Median (IQR) or number (%)[1]			
	Couples with HIV-positive female partner (N = 4872)		Couples with HIV-positive male partner (N = 2659)	
	HIV-1 seropositive woman	HIV-1 seronegative man	HIV-1 seropositive man	HIV-1 seronegative women
Demographic characteristics				
Age, years	29 (25-35)	34 (29-41)	38 (33-45)	32 (27-38)
Education, years	7 (5-10)	8 (6-12)	7 (5-10)	7 (3-9)
Any monthly income	2073 (42.5%)	3634 (74.6%)	2072 (77.9%)	1435 (54.0%)
Couple and behavioural characteristics				
Partners HSV-2 (vs. Partners PrEP)	2081 (42.7%)		952 (35.8%)	
Eastern Africa (vs. southern Africa)[2]	4164 (85.5%)		2347 (88.3%)	
Married	4312 (88.5%)		2483 (93.4%)	
Living together	4561 (93.6%)		2556 (96.1%)	
Duration of partnership, years	5 (2-10)		10 (4-17)	
Number of sex acts, prior month	4 (2-8)		4 (2-8)	
Any unprotected sex acts, prior month	1717 (35.2%)		797 (30.0%)	
Any sex acts with outside partner, prior month	58 (1.2%)	463 (9.5%)	321 (12.1%)	13 (0.5%)
Medical characteristics				
T. vaginalis	627 (12.9%)	244 (5.0%)	75 (2.8%)	230 (8.7%)
N. gonorrhoeae[3]	88 (1.8%)	33 (0.7%)	24 (0.9%)	36 (1.4%)
C. trachomatis[3]	71 (1.5%)	100 (2.1%)	15 (0.6%)	34 (1.3%)
T. pallidum serology	156 (3.3%)	130 (2.7%)	118 (4.5%)	96 (3.7%)
Bacterial vaginosis (Nugent score)	1326 (30.3%)	—	—	673 (26.9%)
Pregnant	370 (7.6%)	—	—	55 (2.1%)
Contraception				
None/condoms only/other	3479 (71.4%)	—	—	1649 (62.0%)
Surgical	193 (4.0%)	—	—	156 (5.9%)
Oral contraceptives	35 (0.7%)	—	—	35 (1.3%)
Intrauterine device	208 (4.3%)	—	—	156 (5.9%)
Implant	115 (2.4%)	—	—	93 (3.5%)
Injectable	842 (17.3%)	—	—	570 (21.4%)
CD4 count, cells/ul	507 (376-686)	—	444 (346-583)	—
Circumcised (men only)	—	2649 (54.4%)	879 (33.1%)	—

[1] Analysis restricted to couples with *T. vaginalis* results for both partners. Some individuals had missing covariate values: age (n=3), education (n=1), duration of partnership (n=12), *N. gonorrhoeae* (n=198), *C. trachomatis* (n=203), *T. pallidum* (n=282), bacterial vaginosis (n=655), and circumcision status (n=2).

[2] Southern African countries were South Africa (n=437), Zambia (n=301), and Botswana (n=282). Eastern African countries were Kenya (n=3287), Uganda (n=2861), Tanzania (n=211), and Rwanda (n=152).

[3] NAATs for detection of *N. gonorrhoeae* and *C. trachomatis*.

Table 2.2 Signs and symptoms of *Trichomonas vaginalis* infection at examination

		Bivariate[1]				Adjusted model[2,3]		
Female partner	N with TV/Total	%	RR	95% CI	*P*	RR	95% CI	*P*
Abnormal vaginal discharge								
Absent	679/6452	10.5	Ref	—	—	Ref	—	—
Present	173/1041	16.6	1.58	1.35-1.84	<0.001	1.50	1.28-1.76	<0.001
Cervical bleeding								
Absent	683/6514	10.5	Ref	—	—	Ref	—	—
Present	169/978	17.3	1.65	1.41-1.92	<0.001	1.35	1.14-1.59	<0.001
Adnexal, cervical, or uterine tenderness								
Absent	818/7278	11.2	Ref	—	—	Ref	—	—
Present	34/213	16.0	1.42	1.04-1.95	0.029	1.46	1.05-2.03	0.025
Male partner	N with TV/Total	%	RR	95% CI	*P*	RR	95% CI	*P*
Urethral discharge								
Absent	318/7517	4.2	Ref	—	—	Ref	—	—
Present	1/13	7.7	1.82	0.28-12.0	0.534	1.37	0.19-9.68	0.754

[1] Some study participants were missing information on signs and symptoms: vaginal discharge (n=38), cervical bleeding (n=39), tenderness (n=40), and urethral discharge (n=1). Among the 852 women with TV who had complete information on the presence of signs and symptoms, 555 were asymptomatic while 231, 53, and 13 women experienced one, two, or three signs and symptoms, respectively.

[2] Adjusted for study, region, *N. gonorrhoeae, C. trachomatis,* bacterial vaginosis (females only), and serology for HSV-2, *T. pallidum*, and HIV-1.

[3] Some study participants had missing covariate values and were excluded from the adjusted model. Among women: *N. gonorrhoeae* (n=169)*, C. trachomatis* (n=173)*,* bacterial vaginosis (n=665), and *T. pallidum* (n=131). Among men: *N. gonorrhoeae* (n=29)*, C. trachomatis* (n=30)*,* and *T. pallidum* (n=151). The number of participants in each adjusted model differed: vaginal discharge (n=6007), cervical bleeding (n=6006), tenderness (n=6005), and urethral discharge (n=7349).

Table 2.3 Correlates of female *Trichomonas vaginalis* infection

			Bivariate model			Multivariable model[1]		
Female Characteristics	N with TV/Total	%	RR	95% CI	*P*	RR	95% CI	*P*
Age, years					0.011[2]			
18-24	223/1625	13.7	Ref	—	—	Ref	—	—
25-34	385/3651	10.6	0.77	0.66-0.90	0.001	0.90	0.76-1.07	0.253
35-44	201/1809	11.1	0.81	0.68-0.97	0.020	0.85	0.68-1.06	0.154
≥45	48/445	10.8	0.79	0.59-1.05	0.108	0.63	0.44-0.89	0.009
Education, years								
<9	626/5336	11.7	Ref	—	—	—	—	—
≥9	231/2195	10.5	0.90	0.78-1.03	0.135	—	—	—
Monthly income								
No earned income	560/4023	13.9	Ref	—	—	Ref	—	—
Earns income	297/3508	8.5	0.61	0.53-0.69	<0.001	0.88	0.76-1.02	0.096
Outside sex partners								
None	842/7460	11.3	Ref	—	—	Ref	—	—
Any	15/71	21.1	1.87	1.19-2.95	0.007	1.78	1.15-2.76	0.009
HIV-1 serostatus								
Negative	230/2659	8.7	Ref	—	—	Ref	—	—
Positive	627/4872	12.9	1.49	1.29-1.72	<0.001	1.19	1.02-1.38	0.023
N. gonorrhoeae								
Negative	810/7238	11.2	Ref	—	—	Ref	—	—
Positive	34/124	27.4	2.45	1.83-3.29	<0.001	1.65	1.23-2.23	0.001
C. trachomatis								
Negative	819/7253	11.3	Ref	—	—	Ref	—	—
Positive	25/105	23.8	2.11	1.49-2.99	<0.001	1.19	0.76-1.86	0.442
T. pallidum serostatus								
Negative	797/7148	11.2	Ref	—	—	—	—	—
Positive	34/252	13.5	1.21	0.88-1.67	0.242	—	—	—
Bacterial vaginosis					<0.001[2]			
Normal	222/3601	6.2	Ref	—	—	Ref	—	—
Intermediate	206/1276	16.1	2.62	2.19-3.13	<0.001	2.04	1.70-2.44	<0.001
Bacterial vaginosis	358/1999	17.9	2.90	2.48-3.40	<0.001	1.96	1.67-2.30	<0.001
Contraception					<0.001[2]			
None/condoms/other	639/5128	12.5	Ref	—	—	Ref	—	—
Surgical	40/349	11.5	0.92	0.68-1.24	0.585	1.07	0.80-1.42	0.658
IUD	3/70	4.3	0.34	0.11-1.04	0.059	0.50	0.18-1.37	0.177
Oral contraceptives	36/364	9.9	0.79	0.58-1.09	0.155	0.95	0.71-1.28	0.736
Implant	21/208	10.1	0.81	0.54-1.22	0.317	1.37	0.87-2.17	0.179
Injectable	118/1412	8.4	0.67	0.56-0.81	<0.001	0.77	0.64-0.94	0.008
Couple Characteristics	N with TV/Total	%	RR	95% CI	*P*	RR	95% CI	*P*
Married to partner								
No	180/736	24.5	Ref	—	—	Ref	—	—
Yes	677/6795	10.0	0.41	0.35-0.47	<0.001	0.74	0.59-0.93	0.011
Living with partner								
No	93/414	22.5	Ref	—	—	Ref	—	—
Yes	764/7117	10.7	0.48	0.39-0.58	<0.001	0.89	0.69-1.13	0.330
Unprotected sex, past month								
None	509/5017	10.2	Ref	—	—	Ref	—	—
Any	348/2514	13.8	1.36	1.20-1.55	<0.001	1.08	0.95-1.24	0.228
Living children								

None	334/1920	17.4	Ref	—	—	Ref	—	—
≥1	523/5610	9.3	0.54	0.47-0.61	<0.001	0.80	0.69-0.92	0.002
Region								
Eastern Africa	629/6511	9.7	Ref	—	—	Ref	—	—
Southern Africa	228/1020	22.4	2.31	2.02-2.65	<0.001	1.19	0.97-1.47	0.097
Study								
PIP	478/3033	15.8	Ref	—	—	Ref	—	—
PrEP	379/4498	8.4	0.53	0.47-0.61	<0.001	0.96	0.82-1.13	0.651
Testing method								
Culture	87/1192	7.3	Ref	—	—	Ref	—	—
NAAT	770/6339	12.2	1.66	1.35-2.06	<0.001	1.07	0.82-1.41	0.613
Male Characteristics	**N with TV/Total**	**%**	**RR**	**95% CI**	***P***	**RR**	**95% CI**	***P***
Age, years					0.007[2]			
18-24	65/476	13.7	Ref	—	—	Ref	—	—
25-34	335/2861	11.7	0.86	0.67-1.10	0.223	1.15	0.90-1.48	0.267
35-44	262/2668	9.8	0.72	0.56-0.93	0.011	0.99	0.75-1.32	0.965
≥45	195/1524	12.8	0.94	0.72-1.22	0.625	1.26	0.93-1.71	0.135
Education, years								
<9	562/4396	12.8	Ref	—	—	Ref	—	—
≥9	294/3134	9.4	0.73	0.64-0.84	<0.001	0.72	0.63-0.82	<0.001
Monthly income								
No earned income	320/1825	17.5	Ref	—	—	Ref	—	—
Earns income	537/5706	9.4	0.54	0.47-0.61	<0.001	0.82	0.71-0.94	0.006
Outside sex partners								
None	757/6746	11.2	Ref	—	—	—	—	—
Any	100/784	12.8	1.14	0.94-1.38	0.198	—	—	—
N. gonorrhoeae								
Negative	845/7445	11.4	Ref	—	—	—	—	—
Positive	10/57	17.5	1.55	0.88-2.72	0.132	—	—	—
C. trachomatis								
Negative	831/7386	11.3	Ref	—	—	Ref	—	—
Positive	24/115	20.9	1.85	1.29-2.66	0.001	1.13	0.75-1.71	0.554
T. pallidum serostatus								
Negative	796/7132	11.2	Ref	—	—	—	—	—
Positive	32/248	12.9	1.16	0.83-1.61	0.389	—	—	—
Circumcision status								
Uncircumcised	492/4001	12.3	Ref	—	—	Ref	—	—
Circumcised	365/3528	10.4	0.84	0.74-0.96	0.008	0.82	0.72-0.94	0.004
T. vaginalis								
Negative	662/7212	9.2	Ref	—	—	Ref	—	—
Positive	195/319	61.1	6.66	5.94-7.46	<0.001	4.70	4.10-5.38	<0.001

[1] N = 6714 for multivariable model after excluding couples with missing values.

[2] The reported *P* value is from a likelihood ratio test across all values of the covariate.

Table 2.4 Correlates of male *Trichomonas vaginalis* infection

			Bivariate model			Multivariable model[1]		
Male Characteristics	N with TV/Total	%	RR	95% CI	*P*	RR	95% CI	*P*
Age, years					<0.001[2]			
18-24	25/476	5.3	Ref	—	—	Ref	—	—
25-34	92/2861	3.2	0.61	0.40-0.94	0.026	0.70	0.44-1.11	0.129
35-44	113/2668	4.2	0.81	0.53-1.23	0.318	1.00	0.62-1.62	0.990
≥45	89/1524	5.8	1.11	0.72-1.71	0.630	0.97	0.58-1.62	0.901
Education, years								
<9	216/4396	4.9	Ref	—	—	Ref	—	—
≥9	103/3134	3.3	0.67	0.53-0.84	0.001	0.78	0.61-1.01	0.057
Monthly income								
No earned income	132/1852	7.2	Ref	—	—	Ref	—	—
Earns income	187/5706	3.3	0.45	0.36-0.56	<0.001	0.83	0.65-1.05	0.125
Outside sex partners								
None	293/6746	4.3	Ref	—	—	—	—	—
Any	26/784	3.3	0.76	0.51-1.13	0.180	—	—	—
HIV-1 serostatus								
Negative	244/4872	5.0	Ref	—	—	Ref	—	—
Positive	75/2659	2.8	0.56	0.44-0.73	<0.001	0.69	0.53-0.90	0.006
N. gonorrhoeae								
Negative	315/7445	4.2	Ref	—	—	—	—	—
Positive	2/57	3.5	0.83	0.21-3.25	0.788	—	—	—
C. trachomatis								
Negative	308/7386	4.2	Ref	—	—	—	—	—
Positive	9/115	7.8	1.88	0.99-3.55	0.053	—	—	—
T. pallidum serostatus								
Negative	296/7135	4.2	Ref	—	—	—	—	—
Positive	10/248	4.0	0.97	0.52-1.80	0.927	—	—	—
Circumcision status								
Uncircumcised	154/4001	3.9	Ref	—	—	—	—	—
Circumcised	165/3528	4.7	1.22	0.98-1.51	0.076	—	—	—
Couple Characteristics	N with TV/Total	%	RR	95% CI	*P*	RR	95% CI	*P*
Married to partner								
No	56/736	7.6	Ref	—	—	Ref	—	—
Yes	263/6795	3.9	0.51	0.39-0.67	<0.001	1.17	0.84-1.64	0.359
Living with partner								
No	23/414	5.6	Ref	—	—	—	—	—
Yes	296/7117	4.2	0.75	0.50-1.13	0.169	—	—	—
Unprotected sex, past month								
None	196/5017	3.9	Ref	—	—	Ref	—	—
Any	123/2514	4.9	1.25	1.00-1.56	0.045	1.00	0.80-1.24	0.965
Living children								
None	103/1920	5.4	Ref	—	—	Ref	—	—
≥1	216/5610	3.9	0.72	0.57-0.90	0.005	1.08	0.85-1.37	0.535
Region								
Eastern Africa	234/6511	3.6	Ref	—	—	Ref	—	—
Southern Africa	85/1020	8.3	2.32	1.83-2.95	<0.001	1.10	0.81-1.51	0.533
Study								

PIP	192/3033	6.3	Ref	—	—	Ref	—	—
PrEP	127/4498	2.8	0.45	0.36-0.56	<0.001	0.72	0.54-0.95	0.021
Testing method								
Culture	16/1192	1.3	Ref	—	—	Ref	—	—
NAAT	303/6339	4.8	3.56	2.16-5.87	<0.001	2.43	1.27-4.65	0.008
Female Characteristics	N with TV/Total	%	RR	95% CI	*P*	RR	95% CI	*P*
Age, years					0.018[2]			
18-24	73/1625	4.5	Ref	—	—	Ref	—	—
25-34	135/3651	3.7	0.82	0.62-1.09	0.171	1.01	0.74-1.37	0.960
35-44	80/1809	4.4	0.98	0.72-1.34	0.921	1.21	0.83-1.75	0.318
≥45	31/445	7.0	1.55	1.03-2.33	0.035	1.47	0.88-2.47	0.146
Education, years								
<9	244/5336	4.6	Ref	—	—	Ref	—	—
≥9	75/2195	3.4	0.75	0.58-0.96	0.025	0.79	0.60-1.04	0.096
Monthly income								
No earned income	222/4023	5.5	Ref	—	—	Ref	—	—
Earns income	97/3508	2.8	0.50	0.40-0.63	<0.001	0.90	0.68-1.19	0.462
Outside sex partners								
None	316/7460	4.2	Ref	—	—	—	—	—
Any	3/71	4.2	1.00	0.33-3.04	0.996	—	—	—
N. gonorrhoeae								
Negative	307/7238	4.2	Ref	—	—	Ref	—	—
Positive	10/124	8.1	1.90	1.04-3.48	0.037	1.15	0.63-2.11	0.647
C. trachomatis								
Negative	313/7253	4.3	Ref	—	—	—	—	—
Positive	4/105	3.8	0.88	0.34-2.32	0.801	—	—	—
T. pallidum serostatus								
Negative	299/7148	4.2	Ref	—	—	—	—	—
Positive	12/252	4.8	1.14	0.65-2.00	0.652	—	—	—
Bacterial vaginosis					<0.001[2]			
Normal	96/3601	2.7	Ref	—	—	Ref	—	—
Intermediate	70/1276	5.5	2.06	1.52-2.78	<0.001	1.14	0.84-1.53	0.395
Bacterial vaginosis	121/1999	6.1	2.27	1.75-2.95	<0.001	1.10	0.85-1.42	0.473
Contraception					0.049[2]			
None/condoms /other	2227/5128	4.4	Ref	—	—	Ref	—	—
Surgical	16/349	4.6	1.04	0.63-1.70	0.890	0.96	0.59-1.56	0.877
IUD	1/70	1.4	0.32	0.05-2.27	0.256	0.66	0.10-4.22	0.664
Oral contraceptives	18/364	5.0	1.12	0.70-1.78	0.643	1.42	0.91-2.22	0.125
Implant	2/208	1.0	0.22	0.05-0.87	0.031	0.25	0.04-1.61	0.145
Injectable	55/1412	3.9	0.88	0.66-1.17	0.385	1.23	0.92-1.65	0.156
T. vaginalis								
Negative	124/6674	1.9	Ref	—	—	Ref	—	—
Positive	195/857	22.8	12.25	9.89-15.16	<0.001	10.09	7.92-12.85	<0.001

[1] N = 6744 for multivariable model after excluding couples with missing values.

[2] The reported *P* value is from a likelihood ratio test across all values of the covariate.

Chapter 3. Associations between schistosomiasis and HIV-1 acquisition risk in four prospective cohorts

Associations between schistosomiasis and HIV-1 acquisition risk in four prospective cohorts

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ABSTRACT

Background Globally, schistosomes infect approximate 200 million people, with 90% of infections in sub-Saharan Africa. Schistosomiasis is hypothesized to increase HIV-1 acquisition risk, and multiple cross-sectional studies reported strong associations between schistosomiasis and HIV-1 infection. We evaluated this hypothesis within four large prospective cohorts.

Methods and Findings We conducted nested case-control analyses within three longitudinal cohorts of heterosexual HIV-1 serodiscordant couples and one female sex worker (FSW) cohort from Kenya and Uganda. Cases HIV-1 seroconverted during prospective follow-up; three controls were selected per case. The presence of circulating anodic antigen in archived serum, collected prior to HIV-1 seroconversion, identified participants with active schistosomiasis; immunoblots determined the schistosome species. Data from serodiscordant couples cohorts were pooled, while the FSW cohort was analyzed separately to allow for appropriate confounder adjustment. We included 245 HIV-1 seroconverters and 713 controls from the serodiscordant couples cohorts and 330 HIV-1 seroconverters and 962 controls from the FSW cohort. The prevalence of active schistosomiasis was 20% among serodiscordant couples and 22% among FSWs. We found no association between schistosomiasis and HIV-1 acquisition risk among males (adjusted odds ratio [aOR] = 0.99, 95% CI 0.59-1.67) or females (aOR = 1.21, 95% CI 0.64-2.30) in serodiscordant couples. Similarly, in the FSW cohort we detected no association (adjusted incidence rate ratio [aIRR] = 1.11, 95% CI 0.83-1.50). Exploring schistosome species-specific effects, there was no statistically significant association between HIV-1 acquisition risk and *S. mansoni* (serodiscordant couples: aOR= 0.90, 95% CI 0.56-1.44; FSW: aIRR = 0.83, 95% CI 0.53-1.20) or *S. haematobium* (serodiscordant couples: aOR = 1.06, 95% CI 0.46-2.40; FSW: aIRR = 1.64, 95% CI 0.93-2.87) infection.

Conclusions Schistosomiasis was not a strong risk factor for HIV-1 acquisition in these four prospective studies among women and men from East Africa.

Abbreviations: CAA, circulating anodic antigen; FSW, female sex workers; SEA, soluble egg antigen.

INTRODUCTION

Schistosomiasis, a parasitic disease caused by the schistosome flatworm, affects approximately 200 million people globally [2], over 90% of whom live in sub-Saharan Africa [53, 54]. In Africa, schistosomiasis is predominantly caused by two schistosome species: *S. haematobium* and *S. mansoni* [55]. Several cross-sectional analyses found strong positive associations between prevalent *S. haematobium* or *S. mansoni* infection and HIV-1 [12-15], which supported the hypothesis that schistosomiasis increases HIV-1 acquisition risk. However, findings from more recent studies are mixed [18, 56, 57], and there remains a need to validate this association in well-powered longitudinal analyses.

Three biological mechanisms have been proposed to explain how schistosomiasis increases susceptibility to HIV-1. Adult female schistosome worms lay hundreds of eggs daily into the venules in which they reside [58, 59], and eggs become deposited into host genital organs. *S. haematobium* and *S. mansoni* have differing pathologies, and eggs are most frequently observed in genital organs of women infected by *S. haematobium*, for whom ova have been found in the cervix of 24-87% of infected women [60]. These ova can remain trapped in the mucosal tissues, causing an influx of immune cells, including CD4+ T-cells targeted by the HIV-1 virus [21]. Trapped ova also induce neovascularization, resulting in mucosal fragility, which may provide HIV-1 direct access to the bloodstream [61, 62]. Additionally, individuals with *S. mansoni* have denser concentrations of HIV-1 co-receptors CCR5 and CXCR4 on CD4+ T-cells, which could increase susceptibility to HIV-1 [63].

In this analysis, we utilized data from four longitudinal studies conducted in Kenya and Uganda. Three of these cohorts enrolled HIV-1 serodiscordant couples while one enrolled female sex workers (FSW). Our objective was to evaluate the hypothesis that schistosomiasis increases HIV-1 acquisition risk.

METHODS

Study population

Longitudinal data from four prospective cohorts were included in this nested case-control analysis. Three cohorts enrolled African heterosexual HIV-1 serodiscordant couples: The Partners in Prevention HSV/HIV Transmission Study [34], the Couples Observational Study [64], and the Partners PrEP Study [35]. These studies were conducted between 2004 and 2012 and enrolled more than 8,500 couples for between 12 and 36 months, with the HIV-negative partner tested for HIV-1 monthly or quarterly. The fourth cohort, the Mombasa Cohort, enrolled FSWs in Mombasa Kenya. This prospective cohort enrolled 3,471 women between 1993 and 2014, with ongoing enrollment and follow-up for as long as women resided in Mombasa. Participants were invited to monthly clinic visits for HIV-1 testing, detailed study procedures have been published elsewhere [65-67].

Eligibility for HIV-1 seroconverters (cases) was consistent across all four cohorts. All participants aged 16 and above who were HIV-1 seronegative at study enrollment, HIV-1 seroconverted during study follow-up, and had a serum or plasma sample collected prior to the HIV-1 seroconversion study visit were included in the analysis. Additionally, inclusion from the Couples Observational Study and Partners in Prevention HSV/HIV Transmission Study was restricted to participants enrolled at sites in Kenya and Uganda, where schistosomiasis is endemic [54]. Serodiscordant couples included in the analysis were enrolled at four sites in Kenya (Kisumu, Nairobi, Eldoret, and Thika) and five sites in Uganda (Kampala, Tororo, Mbale, Kabwohe, and Jinja) while all FSW were enrolled in Mombasa, Kenya.

Three controls were selected per HIV-1 seroconverter, with appropriate sampling methodology used for the serodiscordant couples and FSW cohorts. Since the serodiscordant couples cohorts were all of relatively short duration, with little loss-to-follow-up, controls were frequency-matched to cases based on study, sex, and study randomization arm (Partners PrEP only), as done in previous nested case-control analyses [24, 68]. Once control participants were identified, a study visit was selected for each control, frequency-matched to the timing of the case sample selected for schistosomiasis testing, using one-year time bands. Serum samples from these visits were tested for schistosomiasis and these visits were used to obtain values for time-varying covariates. For the FSW cohort, which by design had variable lengths of participant follow-up, controls were selected using incidence density sampling [69], matching on 2-year periods of study enrollment. Incidence density sampling has the advantage of reducing potential bias caused by

differential loss to follow-up between schistosomiasis infected and uninfected controls since sampling probability is proportional to the amount of cohort follow-up time accrued [70].

All study protocols, including planned analyses for HIV-1 transmission risk factors, were approved by the University of Washington Human Subjects Division as well as ethics review committees at each study site. The study was also reviewed by the CDC, which deemed CDC personnel to be non-engaged as they had no contact with study participants or access to personal identifiers. All study participants provided written informed consent.

Laboratory testing

Schistosomiasis testing was conducted using a three-stage testing algorithm. First, all samples were tested by ELISA using soluble egg antigen (SEA) to detect antischistosomal antibodies [71]. Because the SEA ELISA cannot differentiate between active or resolved infection, SEA-positive samples were tested for the presence of schistosome circulating anodic antigen (CAA) using the SCAA20 assay (detection threshold of 10 pg/ml) [72], which specifically detects active infections [73]. For samples that were SEA and CAA positive, species-specific immunoblots were performed for *S. mansoni* and *S. haematobium* to identify the schistosome species causing infection [55]. Because schistosome antigen levels correlate with worm burden [72], results from the SCAA20 assay were used to classify infection intensity as low (10-99 pg/ml), medium (100-999 pg/ml), or high burden (≥1000 pg/ml), as done by others [74]. SEA and species-specific immunoblot testing was performed by the U.S. Centers for Disease Control and Prevention, while CAA testing was performed at Leiden University Medical Center.

HIV-negative study participants were tested for HIV-1 during each routine study visit. For the serodiscordant couples cohorts, dual rapid HIV-1 antibody tests were performed during clinic visits, with confirmatory HIV-1 enzyme immunoassay and western blot. For the Mombasa cohort, HIV-1 testing was performed via an ELISA, with positive results confirmed by a second ELISA.

Statistical methods

For all cohorts, bivariate and multivariable models were used to assess associations between schistosomiasis and HIV-1 acquisition risk. Data for the three serodiscordant couples cohorts were pooled and analyzed together, while the FSW cohort was analyzed in separate statistical models. The serodiscordant couples and FSW cohorts were analyzed separately to permit adjustment for the complete set of possible confounders collected for each population and because different sampling methodologies were used. For the frequency-matched cases and controls of the serodiscordant couples cohorts, logistic regression models were used to estimate odds ratios. Conditional logistic regression models were used for the FSW cohort's incidence-density matched cases and control to estimate incidence rate ratios. All analyses were done using robust standard errors and were performed using Stata version 13.1 (Stata Corporation, College Station, TX).

For all cohorts, two types of potential confounders were identified prior to the analysis: *a priori* confounders identified through the available literature whose inclusion in all statistical models was pre-determined, as well as a list of potential covariates only to be included if empirically found to be meaningful confounders (>10% change in the effect estimate). The lists of confounders and their definitions differed between the two populations, as the standard approach to covariate adjustment was used for each population. *A priori* confounders were age, sex, and study/site combination for the serodiscordant couples cohorts and age, year of study enrollment, and workplace (a marker of socioeconomic status) for the FSW cohort. Potential confounders were generally factors associated with HIV-1 acquisition risk but whose association with schistosomiasis was inconsistent or unknown. For the serodiscordant couples cohorts, the potential confounders we evaluated were income, education, diagnosis of trichomoniasis, gonorrhea or chlamydia, HSV-2 serostatus, male circumcision status, PrEP study arm, contraceptive use (time-varying), pregnancy status (time-varying), unprotected sex (time-varying), other sex partners (time-varying), and genital ulcer disease (time-varying). For the FSW cohort, potential confounders included education, parity, nationality (Kenyan/other), marital status, vaginal washing practices, unprotected sex (time-varying), number of sex partners (time-varying), contraceptive use (time-varying), gonorrhea (time-varying), trichomoniasis (time-varying), and HSV-2 serostatus (time-varying). None of these additional variables meaningfully (>10%) changed the effect estimates for our primary model for either the serodiscordant couples or FSW cohorts, and thus were not included in multivariable models.

We used analogous statistical models with the same sets of covariates to perform subgroup and sensitivity analyses. In the serodiscordant couples cohort, we performed subgroup analyses by sex, to evaluate specifically the hypothesis that the association between schistosomiasis and HIV-1 acquisition is specific to females. Additionally, we performed sensitivity analyses evaluating associations by schistosome infection intensity and schistosome species. Infection intensity was modeled using indicator variables for each levels of infection; additionally, a test for trend was performed modeling infection intensity levels linearly. Schistosome species were modeled using indicator variables for *S. mansoni*, *S. haematobium,* and infections caused by an undetermined species (CAA positive but tested negative for both species), which may represent recently acquired schistosome infections.

RESULTS

From the 7,026 couples enrolled in the serodiscordant couples cohorts at sites in Kenya and Uganda, we identified 245 individuals who HIV-1 seroconverted (94 from Partners in Prevention HSV/HIV Transmission Study, 13 from Couples Observational Study, and 138 from Partners PrEP) and 713 frequency-matched controls. In the Mombasa FSW Cohort, from 2,160 HIV-1 uninfected participants who enrolled in the cohort, 332 individuals seroconverted, of whom 330 had a blood sample available for schistosomiasis testing and were included in the analysis. 990 control samples were selected, of whom 28 were later excluded due to insufficient sample volume available for schistosomiasis testing, leaving 962 controls included in the analysis. Characteristics of individuals who HIV-1 seroconverted and controls are shown in Table 1. Among HIV-1 seroconverters, samples tested for schistosomiasis were collected a median of 84 days (interquartile range [IQR], 53-92) and 166 days (IQR, 96-424) prior to the seroconversion study visit for individuals in the SDC and FSW cohorts respectively.

In the serodiscordant couples cohorts, 32% (305/958) of samples were antischistosomal (SEA) antibody positive, of whom 64% (194/305) tested positive for schistosome antigens (CAA) indicating an active schistosome infection. In the FSW cohort, 34% (439/1,292) of samples were antischistosomal antibody positive, of whom 66% (290/439) tested antigen positive. Thus, the prevalence of active schistosomiasis was 20% (194/958) within the serodiscordant couples cohorts and 22% (290/1,292) within the FSW cohort.

Additionally, among a 10% sample of antischistosomal antibody negative specimens from all cohorts, only 3% (4/142) tested antigen positive, indicating that the antibody assay was highly sensitive in these populations.

In the serodiscordant couples cohorts, we found no evidence of an association between schistosomiasis and HIV-1 acquisition (Table 2). We estimated the association for all participants (adjusted odds ratio [aOR] = 1.08, 95% confidence interval [CI] 0.73-1.60) and separately for males (aOR = 0.99, 95% CI 0.59-1.67) and females (aOR = 1.21, 95% CI 0.64-2.30). In the FSW cohorts, we also found no evidence of an association between schistosomiasis and HIV-1 acquisition (adjusted incidence rate ratio [aIRR] = 1.11, 95% CI 0.83-1.50). Because some mucosal damage from schistosome ova has been shown to persist years beyond the period of active infection [75], we also evaluated if ever having experienced a schistosome infection increased HIV-1 acquisition risk (using the anti-SEA antibody result), but found no association (Table 3).

Since schistosome antigen levels correlate with worm burden and higher worm burden leads to increased schistosome ova and subsequent mucosal damage, we evaluated associations between schistosomiasis and HIV-1 acquisition stratified by level of infection intensity (Tables 3 & S1). Compared to individuals without evidence of prior schistosome infection, we found no evidence that individuals with high intensity infections faced an increased risk of HIV-1 acquisition in the serodiscordant couples (aOR = 0.96, 95% CI 0.51-1.82) or FSW (aIRR = 0.91, 95% CI 0.58-1.43) cohorts. We also assessed if there was a linear trend of increasing HIV-1 acquisition risk across increasing schistosome infection intensity levels, but did not find an association in either the serodiscordant couples (P = 0.954, females subgroup P = 0.450) or FSW cohorts (P = 0.603).

Lastly, we evaluated if specific schistosome species were associated with HIV-1 acquisition risk (Tables 4 & S2). In the serodiscordant couples cohorts, there was a 4% (36/957) prevalence of *S. haematobium* and 14% (134/957) prevalence of *S. mansoni*. In the FSW cohort the prevalence of *S. haematobium* and *S. mansoni* were 6% (71/1,290) and 18% (233/1,290) respectively. In the serodiscordant couples cohorts we

found no evidence that either *S. mansoni* (aOR = 0.90, 95% CI 0.56-1.44) or *S. haematobium* (aOR = 1.06, 95% CI 0.46-2.40) were associated with HIV-1 acquisition risk. Restricting to female participants, we still identified no association. Similarly, we did not find a statistically significant association between *S. mansoni* (aIRR = 0.83, 95% CI 0.58-1.20) or *S. haematobium* (aIRR = 1.64, 95% CI 0.93-2.87) and HIV-1 acquisition risk in the FSW cohort. Additionally, we stratified positive results for each schistosome species by level of infection intensity, and found no evidence that individuals with high intensity infections caused by *S. mansoni* or *S. haematobium* experienced increased HIV-1 acquisition risk.

DISCUSSION

In this analysis from four large prospective cohort studies from East Africa, we did not find a statistically significant association between schistosomiasis and the risk of HIV-1 acquisition. In both the serodiscordant couples and FSW cohorts, the majority of schistosomiasis was caused by *S. mansoni* infection, and the lack of an association between *S. mansoni* and HIV-1 acquisition risk was consistent as we explored the data across multiple subgroups: female sex workers, serodiscordant couples, male, females, and by levels of infection intensity. These results suggest that *S. mansoni* is not a major driver of the HIV-1 epidemic throughout sub-Saharan Africa.

We did not identify a statistically significant association between *S. haematobium* and HIV-1 acquisition risk, overall or in sex-stratified analyses. One hypothesis in the field has been that *S. haematobium* increases HIV-1 acquisition risk for women. In our analyses, the point estimate for FSWs with *S. haematobium* was 1.64, but this was not statistically significant. Our results across all subgroups suggest that schistosomiasis is not associated with a large increased risk of HIV-1 acquisition (RR ≥3), as suggested by some studies [12, 14, 18]; very large studies would be needed to have sufficient statistical power to rule out whether *S. haematobium* is associated with a more moderate increase in HIV-1 acquisition risk among women.

Little evidence suggests schistosomiasis increases HIV-1 risk in men. Three of the four original cross-sectional studies that found an association between schistosomiasis and HIV-1 included only female participants [12-15]. A recent longitudinal analysis found an association between *S. mansoni* and HIV-1

acquisition, but it was specific to women, with no association observed among men [18]. Most cross-sectional analyses that included both sexes found no evidence of an association [76-78], as did a recent study which included only men [79]. Our results are consistent with these findings.

Though the existing evidence suggesting an association between schistosomiasis and HIV-1 acquisition among women is stronger than among men [12-14], findings have been mixed for both *S. mansoni* and *S. haematobium* [80], and our results are not consistent with a recent study that found *S. mansoni* infection associated with increased HIV-1 acquisition risk in Tanzania [18]. A challenge when evaluating the overall strength of existing evidence is that many published analyses did not present separate subgroup analyses for males and females. This was the case for two recent longitudinal studies [56, 57] and previously published cross-sectional studies that found no association [76-78]; the proportion of female participants in these study populations varied from 28 to 75%. Though the existing epidemiological evidence suggesting associations between HIV-1 acquisition risk and schistosomiasis is inconsistent for both schistosome species, evidence for a biological mechanism is much stronger for *S. haematobium*. *S. haematobium* ova-induced genital damage has been well documented, and genital bleeding and blood in urine are commonly observed symptoms of individuals infected by *S. haematobium* [22, 81, 82].

Some past studies that observed associations between schistosomiasis and HIV-1 acquisition intentionally selected a study population with very high schistosome infection burdens, such as communities living adjacent to schistosome-infected bodies of water. In contrast, participants in the present studies were selected because of their elevated risk of HIV-1 acquisition and were enrolled at 10 locations across Kenya and Uganda. The prevalence of schistosomiasis in our study populations was similar to the estimated national prevalence for Kenya and Uganda, 23% and 20% respectively [83]. Thus, the schistosome burden of our study populations should be generalizable to the majority of individuals with schistosomiasis in sub-Saharan Africa. Additionally, we found no evidence of increased HIV-1 acquisition risk among individuals with high intensity infections, who would be expected to have the most schistosome ova-induced genital damage.

Strengths of our analysis include the use of two distinct high-risk populations. The serodiscordant couples and FSW cohorts each independently included more HIV-1 seroconverters than any prior longitudinal

analysis; thus, this manuscript includes the two best-powered analyses conducted to-date. The large amount of demographic, behavioral, and clinical information collected from study participants enabled thorough adjustment for potential confounders. The schistosomiasis testing algorithm differentiated between active and previous schistosome infections. This permitted us to conduct secondary analyses evaluating if individuals with active or previous schistosome infections faced increased risk of HIV-1 acquisition, addressing the possibility that damage from schistosome ova and increased HIV-1 susceptibility persist beyond the period of active infection [75]. Additionally, the antigen results allowed us to perform sub-analyses by schistosome infection intensity, while the species-specific immunoblots enabled us to evaluate associations for each schistosome species.

Since we utilized data from previously conducted cohort studies, one limitation is that we lack data on clinical manifestations of schistosome infection among our study population. Through genital examinations, damage from schistosome ova can be identified most specifically as “grainy sandy patches” [22], and it is possible that only women with these patches have increased HIV-1 acquisition risk. However, all past analyses that observed associations between schistosomiasis and HIV-1 relied on diagnostic testing to identify their schistosome-infected populations. The single cross-sectional study that evaluated associations between grainy sandy patches and HIV-1 did not find a statistically significant association [13]. Additionally, to address this limitation, we performed sub-analyses by level of infection intensity, since participants with high-intensity infections might be expected to have the most severe genital damage from schistosome ova. A second limitation is that though the SCAA20 assay used to identify active infections has a high sensitivity (80-95%), it lacks the sensitivity to detect very low-burden infections. However, two of the five studies that found an association between schistosomiasis and HIV-1 relied on either the SCAA20 [12] or SEA ELISA assays [15] we utilized, and we expect that any individuals with low-burden infections misclassified by our assay would have limited schistosome ova-induced genital damage.

In conclusion, we found no statistically significant effect of schistosome infection on HIV-1 acquisition among this diverse population from East Africa. Our evidence was robust that *S. mansoni* was not associated with an increased risk of HIV-1 acquisition, in men or women. In the FSW cohort, but not the serodiscordant couples cohorts, *S. haematobium* was associated with a point estimate of elevated HIV-1

risk but this was not statistically significant and there was no dose-response effect. Regardless of whether or not schistosomiasis impacts HIV-1 acquisition risk, reducing the morbidity and mortality caused by schistosome infections necessitates the continued expansion of preventive treatment for schistosomiasis [84].

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Contributors: AFB, JMB, CC, and RVB initially conceived of the study, with WES, GJD, PLAMC, AAS, SMN, NRM, and RSM contributing to the study design. AFB, JMB, WES, GJD, PLAMC, NRM, CC, AM, RSM, and RVB supported acquiring and interpreting the data, while AFB, JMB, ASS, and RVB were involved in the analysis. The manuscript was prepared by AFB, and all authors contributed to revision of the manuscript and approved the final version.

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Disclaimer: the findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC.

Table 3.1 Participant characteristics

	Serodiscordant Couples Cohorts		FSW Cohort	
	HIV Seroconverted (N = 245)	Controls (N = 713)	HIV Seroconverted (N = 330)	Controls (N = 962)
Age[1]				
16-24	52 (21%)	91 (13%)	72 (22%)	205 (21%)
25-34	120 (50%)	327 (46%)	176 (53%)	453 (47%)
≥35	73 (30%)	295 (41%)	82 (25%)	304 (32%)
Sex				
Female	128 (52%)	266 (37%)	330 (100%)	962 (100%)
Male	117 (48%)	447 (63%)	—	—
Education[2]				
<9 years	167 (68%)	437 (61%)	212 (64%)	607 (63%)
≥9 years	78 (32%)	276 (39%)	118 (36%)	355 (37%)
Married[3]				
Yes	238 (97%)	695 (97%)	176 (53%)	512 (53%)
No	7 (3%)	18 (3%)	154 (47%)	450 (47%)
Enrollment location[4]				
Kenya	122 (50%)	381 (53%)	330 (100%)	962 (100%)
Uganda	123 (50%)	332 (47%)	—	—
Any unprotected sex[5]				
Yes	72 (30%)	139 (20%)	162 (49%)	447 (46%)
No	172 (70%)	572 (80%)	168 (51%)	515 (54%)
Number of sex partners[5]				
≤1	219 (91%)	626 (88%)	259 (78%)	729 (76%)
>1	22 (9%)	72 (10%)	71 (22%)	233 (24%)
Sexually transmitted infections[6]				
Yes	27 (11%)	57 (8%)	56 (17%)	77 (9%)
No	218 (89%)	656 (92%)	270 (83%)	769 (91%)
Serodiscordant couples cohort				
Partners HSV/HIV Transmission Study	94 (38%)	262 (37%)	—	—
Couples Observational Study	13 (5%)	39 (5%)	—	—
Partners PrEP Study	138 (56%)	412 (58%)	—	—
Workplace				
Nightclub	—	—	41 (12%)	249 (26%)
Bar/Other	—	—	289 (88%)	713 (74%)

[1] For the serodiscordant couples cohorts, age at enrollment was assessed since the longest period of study enrollment was three years. For the FSW cohort, age was time-varying.

[2] Years of education at time of cohort enrollment.

[3] For the serodiscordant couples cohorts, marital status at the time of study enrollment was assessed. For the FSW cohort, marital status at enrollment was categorized as ever married vs. never married because few participants were married (18/1,292).

[4] For the serodiscordant couples cohorts, cases and controls were enrolled at four sites in Kenya [Kisumu (n=192), Nairobi (n=119), Eldoret (n=100), and Thika (n=92)] and five sites in Uganda [Kampala (n=210), Tororo (n=79), Mbale (n=71), Kabwohe (n=59), and Jinja (n=36)]. Enrollment for the FSW cohort was done in Mombasa, Kenya.

[5] For the serodiscordant couples cohorts, sexual behaviors were assessed over the prior month. Some individuals had missing values for unprotected sex (n=3) and number of sexual partners (n=19). For the

FSW cohort, average sexual behaviors were calculated for each year of cohort follow-up. For both cohorts, sexual behaviors were assessed at all study visits and was time-varying.

[6] For the serodiscordant couples cohorts, testing for sexually transmitted infections (trichomoniasis, gonorrhea, and chlamydia) was done at enrollment. For the FSW cohort, sexually transmitted infection testing (trichomoniasis and gonorrhea) occurred at each study visit and was time-varying, and some individuals lacked test results (n=116).

Table 3.2 Associations between schistosomiasis and the risk of HIV-1 acquisition

ACTIVE SCHISTOSOME INFECTION								
			Bivariate[1]			Multivariable model[2]		
Serodiscordant couples cohorts	**HIV SC/Total (%)**		**OR**	**95% CI**	***P***	**aOR**	**95% CI**	***P***
All participants								
No schistosomiasis	193/764	(25)	Ref	—	—	Ref	—	—
Schistosomiasis[3]	52/194	(27)	1.08	0.76-1.55	0.660	1.08	0.73-1.60	0.700
Males								
No schistosomiasis	86/425	(20)	Ref	—	—	Ref	—	—
Schistosomiasis[3]	31/139	(22)	1.13	0.71-1.80	0.602	0.99	0.59-1.67	0.981
Females								
No schistosomiasis	107/339	(32)	Ref	—	—	Ref	—	—
Schistosomiasis[3]	21/55	(38)	1.34	0.74-2.42	0.333	1.21	0.64-2.30	0.552
FSW cohort	**HIV SC/Total (%)**		**IRR**	**95% CI**	***P***	**aIRR**	**95% CI**	***P***
Females								
No schistosomiasis	248/1,002	(25)	Ref	—	—	Ref	—	—
Schistosomiasis[3]	82/290	(28)	1.20	0.89-1.61	0.224	1.11	0.83-1.50	0.478
ACTIVE OR PRIOR SCHISTOSOME INFECTION								
			Bivariate[1]			Multivariable model[2]		
Serodiscordant couples cohorts	**HIV SC/Total (%)**		**OR**	**95% CI**	***P***	**aOR**	**95% CI**	***P***
All participants								
Antibody (anti-SEA) negative	172/653	(26)	Ref	—	—	Ref	—	—
Antibody (anti-SEA) positive	73/305	(24)	0.88	0.64-1.21	0.427	0.87	0.62-1.24	0.444
Males								
Antibody (anti-SEA) negative	77/355	(22)	Ref	—	—	Ref	—	—
Antibody (anti-SEA) positive	40/209	(19)	0.85	0.56-1.31	0.471	0.76	0.46-1.23	0.257
Females								
Antibody (anti-SEA) negative	95/298	(32)	Ref	—	—	Ref	—	—
Antibody (anti-SEA) positive	33/96	(34)	1.12	0.69-1.82	0.650	1.04	0.62-1.74	0.891
FSW cohort	**HIV SC/Total (%)**		**IRR**	**95% CI**	***P***	**aIRR**	**95% CI**	***P***
Females								
Antibody (anti-SEA) negative	205/853	(24)	Ref	—	—	Ref	—	—
Antibody (anti-SEA) positive	125/439	(28)	1.26	0.97-1.65	0.088	1.18	0.90-1.55	0.235

[1] The serodiscordant couples cohorts were adjusted for age, sex, and study/site combination. Male/female subgroup models did not adjust for sex.

[2] The FSW cohort was matched on year of study enrollment (two year bands) and adjusted for age and workplace.

[3] Schistosomiasis: samples with detectable antischistosomal antibodies (anti-SEA) and schistosome antigens (CAA). Samples with negative antischistosomal antibodies or antigen results were classified as not having schistosomiasis.

Table 3.3 Associations between schistosomiasis infection intensity and the risk of HIV-1 acquisition

	Male and Female				Female			
Serodiscordant couples cohorts	**HIV SC/Total (%)**	**aOR[1]**	**95% CI**	***P***	**HIV SC/Total (%)**	**aOR[1]**	**95% CI**	***P***
No infection[3]	172/653 (26)	Ref	—	—	95/298 (32)	Ref	—	—
Past but not current	21/111 (19)	0.66	0.39-1.12	0.127	12/41 (29)	0.86	0.41-1.80	0.681
Low intensity	14/54 (26)	1.00	0.52-1.94	0.996	4/15 (27)	0.69	0.23-2.08	0.506
Moderate intensity	21/75 (28)	1.07	0.61-1.89	0.816	9/23 (39)	1.22	0.48-3.06	0.676
High intensity	17/65 (26)	0.96	0.51-1.82	0.901	8/17 (47)	1.82	0.59-5.61	0.294
FSW cohort	**HIV SC/Total (%)**	**aIRR[2]**	**95% CI**	***P***	**HIV SC/Total (%)**	**aIRR[2]**	**95% CI**	***P***
No infection[3]	—	—	—	—	205/853 (24)	Ref	—	—
Past but not current	—	—	—	—	43/149 (29)	1.24	0.84-1.83	0.280
Low intensity	—	—	—	—	20/61 (33)	1.47	0.83-2.63	0.188
Moderate intensity	—	—	—	—	32/111 (29)	1.28	0.80-2.07	0.305
High intensity	—	—	—	—	30/118 (25)	0.91	0.58-1.43	0.684

[1] The serodiscordant couples cohorts were adjusted for age, sex, and study/site combination. Female subgroup models did not adjust for sex.

[2] The FSW cohort was matched on year of study enrollment (two-year bands) and adjusted for age and workplace.

[3] Definition of infection intensity categories: No infection (anti-SEA negative), past infection (anti-SEA positive & CAA <10 pg/ml), low intensity (anti-SEA positive & CAA 10-99 pg/ml), medium intensity (anti-SEA positive & CAA 100-999 pg/ml), and high intensity (anti-SEA positive & CAA ≥1000 pg/ml).

Table 3.4 Schistosome species-specific associations with the risk of HIV-1 acquisition

	Male and Female					Female				
Serodiscordant couples cohorts [1]	**HIV SC/Total (%)**		**aOR [2]**	**95% CI**	***P***	**HIV SC/Total (%)**		**aOR[2]**	**95% CI**	***P***
Species-specific associations with HIV-1 acquisition risk[3]										
No active infection	193/764	(25)	Ref	—	—	107/339	(32)	Ref	—	—
S. mansoni	31/134	(23)	0.90	0.56-1.44	0.660	13/36	(36)	1.23	0.56-2.68	0.611
S. haematobium	10/36	(28)	1.06	0.46-2.40	0.898	2/9	(22)	0.44	0.08-2.29	0.328
Undetermined species	15/42	(36)	1.51	0.76-3.02	0.238	6/13	(46)	1.47	0.45-4.81	0.523
S. mansoni infection intensity and HIV-1 acquisition risk[4]										
No active *S. mansoni* infection	214/823	(26)	Ref	—	—	115/357	(32)	Ref	—	—
Low intensity infection	7/34	(21)	0.76	0.32-1.83	0.546	2/9	(22)	0.53	0.12-2.29	0.394
Moderate intensity infection	14/53	(26)	1.07	0.55-2.07	0.846	5/14	(36)	1.02	0.31-3.33	0.973
High intensity infection	10/47	(21)	0.76	0.36-1.61	0.470	6/13	(46)	1.95	0.54-7.08	0.312
S. haematobium infection intensity and HIV-1 acquisition risk[4]										
No active *S. haematobium* infection	235/921	(26)	Ref	—	—	126/384	(33)	Ref	—	—
Low intensity infection	2/7	(29)	0.97	0.16-5.78	0.975	0/2	(0)	—	—	—
Moderate intensity infection	5/16	(31)	1.18	0.38-3.62	0.778	1/4	(25)	0.51	0.06-4.57	0.548
High intensity infection	3/13	(23)	0.77	0.19-3.07	0.707	1/3	(33)	0.87	0.11-6.74	0.892
FSW cohort[1]	**HIV SC/Total (%)**		**aIRR [2]**	**95% CI**	***P***	**HIV SC/Total (%)**		**aIRR[2]**	**95% CI**	***P***
Species-specific associations with HIV-1 acquisition risk[3]										
No active infection	—		—	—	—	248/997	(25)	Ref	—	—
S. mansoni	—		—	—	—	57/232	(25)	0.83	0.58-1.20	0.326
S. haematobium	—		—	—	—	25/70	(36)	1.64	0.93-2.87	0.087
Undetermined species	—		—	—	—	11/26	(42)	1.89	0.88-4.08	0.104
S. mansoni infection intensity and HIV-1 acquisition risk[4]										
No active *S. mansoni* infection	—		—	—	—	271/1,052	(26)	Ref	—	—
Low intensity infection	—		—	—	—	10/40	(25)	0.95	0.45-2.02	0.899
Moderate intensity infection	—		—	—	—	23/88	(26)	1.09	0.63-1.88	0.767
High intensity infection	—		—	—	—	24/104	(23)	0.75	0.47-1.22	0.248
S. haematobium infection intensity and HIV-1 acquisition risk[4]										
No active *S. haematobium* infection	—		—	—	—	303/1,214	(25)	Ref	—	—
Low intensity infection	—		—	—	—	6/19	(32)	1.28	0.45-3.61	0.640
Moderate intensity infection	—		—	—	—	10/25	(40)	1.90	0.78-4.60	0.157
High intensity infection	—		—	—	—	9/26	(35)	1.30	0.55-3.10	0.549

[1] Due to insufficient sample volumes, three antigen-positive samples were excluded from species testing: serodiscordant couples cohorts (n=1) and FSW cohort (n=2). In the FSW cohort, both samples with insufficient volume were seroconverters, leading to the exclusion of their matched controls (n=6). Thus, 957 individuals were included in the serodiscordant couples species analyses and 1284 individuals were included in the FSW species analyses. In the serodiscordant couples cohorts, 19 individuals (4 seroconverters) were co-infected with *S. mansoni* and *S. haematobium* while in the FSW cohort 41 individuals (13 seroconverters) were co-infected with both species.

[2] The serodiscordant couples cohorts were adjusted for age, sex, and study/site combination. Female subgroup models did not adjust for sex. The FSW cohort was matched on year of study enrollment (two-year bands) and adjusted for age and workplace.

[3] Definition of species-specific categories: No active infection (anti-SEA negative or CAA <10 pg/ml), *S. mansoni* infection (anti-SEA positive, CAA ≥10 pg/ml, and *S. mansoni* immunoblot positive), *S. haematobium* infection (anti-SEA positive, CAA ≥10 pg/ml, and *S. haematobium* immunoblot positive) and undetermined species (anti-SEA positive, CAA ≥10 pg/ml, and both *S. haematobium* and *S. mansoni* immunoblot negative).

[4] Definition of infection intensity categories: No active infection (anti-SEA negative or CAA <10 pg/ml or species immunoblot negative), low intensity (anti-SEA positive, species immunoblot positive, and CAA 10-99 pg/ml), medium intensity (anti-SEA positive, species immunoblot positive, and CAA 100-999 pg/ml), and high intensity (anti-SEA positive, species immunoblot positive, and CAA ≥1000 pg/ml).

Table S3.1 Associations between schistosomiasis infection intensity and the risk of HIV-1 acquisition among men

	Male			
Serodiscordant couples cohorts	**HIV SC/Total (%)**	**aOR[2]**	**95% CI**	***P***
No evidence of infection[1]	77/355 (22)	Ref	—	—
Past infection	9/70 (13)	0.51	0.23-1.13	0.098
Low intensity	10/39 (26)	1.10	0.49-2.44	0.823
Moderate intensity	12/52 (23)	0.92	0.43-1.97	0.839
High intensity	9/48 (19)	0.70	0.30-1.68	0.430

[1] Adjusted for age, and study/site combination.

[2] Definition of infection intensity categories: No evidence of infection (anti-SEA negative), past infection (anti-SEA positive & CAA <10 pg/ml), low intensity (anti-SEA positive & CAA 10-99 pg/ml), medium intensity (anti-SEA positive & CAA 100-999 pg/ml), and high intensity (anti-SEA positive & CAA ≥1000 pg/ml).

Table S3.2 Schistosome species-specific associations with the risk of HIV-1 acquisition among men

		Male		
Serodiscordant couples cohorts	**HIV SC/Total (%)**	**aOR[1]**	**95% CI**	***P***
Species-specific associations with HIV-1 acquisition risk[2]				
No active infection	86/425 (20)	Ref	—	—
S. mansoni	18/98 (18)	0.73	0.39-1.36	0.317
S. haematobium	8/27 (30)	1.47	0.54-4.00	0.454
Undetermined species	9/29 (31)	1.58	0.68-3.67	0.291
S. mansoni infection intensity and HIV-1 acquisition risk[3]				
No active *S. mansoni* infection	99/466 (21)	Ref	—	—
Low intensity infection	5/25 (20)	0.83	0.28-2.44	0.739
Moderate intensity infection	9/39 (23)	1.01	0.44-2.31	0.980
High intensity infection	4/34 (12)	0.42	0.13-1.29	0.130
S. haematobium infection intensity and HIV-1 acquisition risk[3]				
No active *S. haematobium* infection	109/537 (20)	Ref	—	—
Low intensity infection	2/5 (40)	1.79	0.27-11.9	0.547
Moderate intensity infection	4/12 (33)	1.43	0.41-5.00	0.578
High intensity infection	2/10 (20)	0.81	0.12-5.58	0.834

[1] Adjusted for age and study/site combination.

[2] Definition of species-specific categories: No active infection (anti-SEA negative or CAA <10 pg/ml), *S. mansoni* infection (anti-SEA positive, CAA ≥10 pg/ml, and *S. mansoni* immunoblot positive), *S. haematobium* infection (anti-SEA positive, CAA ≥10 pg/ml, and *S. haematobium* immunoblot positive) and undetermined species (anti-SEA positive, CAA ≥10 pg/ml, and both *S. haematobium* and *S. mansoni* immunoblot negative).

[3] Definition of infection intensity categories: No active infection (anti-SEA negative or CAA <10 pg/ml or species immunoblot negative), low intensity (anti-SEA positive, species immunoblot positive, and CAA 10-99 pg/ml), medium intensity (anti-SEA positive, species immunoblot positive, and CAA 100-999 pg/ml), and high intensity (anti-SEA positive, species immunoblot positive, and CAA ≥1000 pg/ml).

Chapter 4. Effects of schistosomiasis on HIV-1 plasma and genital set point viral loads

Effects of schistosomiasis on HIV-1 plasma and genital set point viral loads

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ABSTRACT

Background: Many regions of sub-Saharan Africa experience a high prevalence of both schistosomiasis and HIV-1, leading to frequent coinfection. Higher plasma HIV-1 viral loads are associated with faster disease progression and genital HIV-1 loads are a primary determinant of HIV-1 transmission risk. We hypothesized that schistosome infection would be associated with higher HIV-1 viral loads in plasma and genital samples.

Methods/Principal Findings: We utilized data from individuals who HIV-1 seroconverted while enrolled in one of four prospective cohort studies. Plasma and genital viral loads collected 4-24 months after the estimated date of HIV-1 acquisition, but prior to antiretroviral therapy initiation, were included. Detection of circulating anodic antigen in archived blood samples, collected prior to HIV-1 seroconversion, identified participants with active schistosomiasis; immunoblots determined the schistosome species causing infection. Our analysis included 370 HIV-1 seroconverters with plasma viral load results, of whom 82 (22%) had schistosomiasis. We did not find a statistically significant association between schistosomiasis and higher HIV-1 set point plasma viral loads (-0.17 $\log_{10}$ copies/ml, 95% CI -0.38 to 0.03); *S. mansoni* infection was associated with a lower set point (-0.34 $\log_{10}$ copies/ml, 95% CI -0.58 to -0.09). We found no association between schistosomiasis and vaginal (+0.07 $\log_{10}$ copies/swab, 95% CI -0.20 to 0.34) or cervical (+0.11 $\log_{10}$ copies/swab, 95% CI -0.17 to 0.39) set point viral loads; *S. haematobium* infection was associated with lower cervical viral loads (-0.59 $\log_{10}$ copies/swab, 95% CI -1.11 to -0.06).

Conclusions/Significant: These results do not support the hypotheses that schistosome coinfection increases plasma or genital HIV-1 viral loads.

Keywords: *Schistosoma mansoni*; *Schistosoma haematobium*; HIV infections/epidemiology; viral load; Africa

INTRODUCTION

Schistosomiasis is a parasitic disease affecting approximately 200 million individuals globally [2]. With 90% of schistosome infections occurring in sub-Saharan Africa [53, 54], there is significant geographical overlap between areas with high schistosomiasis and HIV-1 prevalence [61]. Other HIV-1 coinfections have been found to increase HIV-1 transmission risk or disease progression [9, 85, 86], and these facts led to the hypothesis that schistosome coinfection plays a role in driving the HIV-1 epidemic in sub-Saharan Africa.

Some evidence has suggested that schistosome infection increases the rate of systemic and genital HIV-1 viral replication, potentially resulting in accelerated rates of disease progression and increased transmission risk [87]. A recent study in Tanzania found higher set point viral load levels among individuals with schistosomiasis [18]. Animal model studies found that schistosome-infected macaques had higher viral loads after SHIV acquisition [19, 20]. One mechanism proposed to explain these associations is that individuals with schistosomiasis have denser concentrations of HIV co-receptors CCR5 and CXCR4 on CD4+ T-cells, which could ease cell-to-cell spread of HIV-1, promoting viral replication and disease progression [63]. Additionally, female schistosomes lay hundreds of eggs per day, which can become deposited in host female genital organs, triggering an inflammatory response with recruitment of leukocytes to the genital epithelium [88-90]. Higher concentrations of leukocytes in the genital epithelium of women have been associated with increased HIV-1 genital viral loads [91]. Through this mechanism, schistosome coinfection has been hypothesized to increase HIV-1 genital viral loads [88, 92, 93], a primary determinant of HIV-1 transmission risk [8].

Using data obtained from individuals in Kenya and Uganda who acquired HIV-1, our objective was to evaluate if schistosome infections were associated with HIV-1 viral loads during the set point period 4-24 months after HIV-1 acquisition, when both plasma and genital HIV-1 viral load levels are relatively stable [94]. Two schistosome species cause schistosomiasis in the study area: *S. haematobium* and *S. mansoni*. We hypothesized that individuals infected with either species would have higher viral loads in plasma

samples. Because the two species have differing pathologies, with *S. haematobium* eggs more often found in the female genital tract [60, 95], we hypothesized a positive association between *S. haematobium* infection and genital viral loads, with an attenuated positive association among individuals infected by *S. mansoni*.

METHODS

Study population

We utilized data obtained from individuals who experienced HIV-1 seroconversion while enrolled in one of four prospective cohort studies conducted in Kenya and Uganda. Three of these cohorts enrolled heterosexual HIV-1 serodiscordant couples: the Partners in Prevention HSV/HIV Transmission Study [34], the Couples Observational Study [64], and the Partners PrEP Study [35]. In total, these three cohorts followed more than 8,500 couples for 12 to 36 months, and 297 individuals seroconverted during follow-up. The fourth study was the Mombasa Cohort, an open cohort of female sex workers that began enrollment in 1993; through 2014, 3,471 women had enrolled and 332 had experienced HIV-1 seroconversion.

Individuals who HIV-1 seroconverted during study follow-up and who had a blood sample collected prior to seroconversion available for schistosomiasis testing were eligible for the present analysis. In addition, for the Partners in Prevention HSV/HIV Transmission Study and Couples Observational Study, we only included participants enrolled at study sites in Kenya and Uganda, where schistosomiasis is endemic [54]. All HIV-1 seroconverters in the four cohorts were invited to continue their cohort enrollment. Seroconverters in the serodiscordant couples cohorts were invited to attend quarterly study visits, while seroconverters in the Mombasa Cohort were invited to monthly visits; at these visits a blood sample was collected to measure plasma viral loads. Additionally, women in the Mombasa Cohort had vaginal and cervical swabs collected to measure genital viral loads (study procedures described previously [96]).

Schistosomiasis and viral load testing

Schistosome infections were identified via a three-stage testing algorithm. Samples collected prior to HIV-1 seroconversion were initially tested by soluble egg antigen (SEA) ELISA to detect antischistosomal

antibodies [71]. Since antibodies persist beyond the period of active infection, SEA ELISA-positive samples were tested for circulating anodic antigen (CAA) in serum using the SCAA20 assay (detection threshold of 10 pg/ml) [72]; CAA becomes undetectable within two weeks after successful treatment with praziquantel [73]. To identify the schistosome species causing infection, CAA-positive samples had species-specific immunoblots performed for *S. mansoni* and *S. haematobium* [55]. SEA ELISAs and species-specific immunoblots were performed by the Parasitic Diseases Branch of the U.S. Centers for Disease Control and Prevention (CDC) and CAA testing was performed at Leiden University Medical Center.

The assay used to measure plasma HIV-1 RNA levels varied across studies (Partners HSV/HIV: COBAS AmpliPrep/COBAS TaqMan HIV-1 RNA assay, version 1.0 [Roche Diagnostics]; Couples Observational Study and Partners PrEP Study: Abbott m2000 Real-Time HIV-1 RNA assay [Abbott]; Mombasa Cohort: Gen-Probe HIV-1 viral load assay [Gen-Probe Incorporated]). Genital swabs collected from the Mombasa Cohort were eluted in 1 ml of buffer, and the concentration of HIV-1 RNA was reported as HIV-1 copies/swab. Quantification of genital HIV-1 RNA was conducted using the Gen-Probe HIV-1 viral load assay (Gen-Probe Incorporated).

Date of HIV-1 infection and HIV-1 set point

We estimated the date of HIV-1 acquisition using both serology and plasma viral load results. Study participants were tested for HIV-1 seroconversion at all routine study visits (monthly or quarterly). For the serodiscordant couples cohorts, dual rapid HIV-1 antibody tests were performed, followed by a confirmatory HIV-1 enzyme immunoassay and Western blot. For the Mombasa Cohort, HIV-1 ELISA testing was performed, with positive results confirmed by a second ELISA. Once a positive serology was confirmed, HIV-1 RNA levels were measured in plasma samples collected at the seroconversion visit and preceding visits until a visit prior to HIV-1 acquisition was identified.

For individuals with detectable HIV-1 RNA prior to HIV-1 seroconversion, we estimated the date of HIV-1 acquisition to be 17 days prior to the date of the first positive HIV-1 RNA result. For those whose first detectable HIV-1 RNA occurred at the seroconversion visit, the date of HIV-1 infection was estimated to be at the midpoint between the last seronegative and first seropositive visit. For this study we excluded

participants without detectable plasma HIV-1 RNA prior to seroconversion who had >1 year between their last seronegative and first seropositive visit, since those individuals' date of HIV-1 acquisition could not be estimated with adequate precision.

We defined the set point period as being between 4-24 months after HIV-1 infection. Thus, we included all eligible plasma or genital viral load samples collected 4-24 months after the estimated date of HIV-1 acquisition. We excluded any samples collected after the participant reported initiating antiretroviral therapy (ART); information on ART use was collected at all study visits. This approach is consistent with previous analyses conducted with these cohorts [25, 97].

Statistical methods

We $\log_{10}$-transformed plasma and genital HIV-1 RNA concentrations to approximate a normal distribution. Samples with undetectable RNA levels were set equal to half the lower limit of quantification (120 copies/ml for plasma and 25 copies/swab for genital samples). Linear mixed-effects models with a random intercept for each individual evaluated if schistosomiasis was associated with plasma or genital set point viral load levels. Multivariable models evaluating set point plasma viral loads adjusted for a set of *a priori* confounders identified through a review of the literature: age [98], sex [99, 100], study, and four year bands of calendar year (Mombasa Cohort only, to account for time trends in HIV-1 transmission in that community) [98]. Multivariable models for genital viral loads included this same set of confounders with additional adjustment for plasma viral load levels obtained at the same study visit as genital viral loads. We used analogous statistical models with the same sets of covariates to perform subgroup analyses by schistosome species. We included indicator variables for *S. mansoni*, *S. haematobium*, and infections caused by an undetermined species (CAA positive but negative immunoblot results for both species) into a single statistical model, with uninfected individuals (CAA negative results) as the reference group. Additionally, to address the possibility that schistosome infection status changed over time, we performed a sensitivity analysis, restricting the selection criteria to include only samples collected 4-12 months after HIV-1 infection. All analyses were conducted using Stata version 13.1 (Stata Corporation, College Station, TX).

Human subjects research

All study protocols, which included provisions for future analyses of HIV-1 transmission risk factors, were approved by the University of Washington Human Subjects Division as well as ethics review committees at each study site. The study was also reviewed by the CDC, which deemed CDC personnel to be non-engaged as they had no contact with study participants or access to personal identifiers. All study participants provided written informed consent.

RESULTS

A total of 245 Kenyan and Ugandan participants from the three serodiscordant couples cohorts and 332 individuals from the Mombasa Cohort experienced HIV-1 seroconversion, and all but two individuals had a pre-seroconversion blood sample available for schistosomiasis testing. From these 575 individuals, 370 (64%) had at least one plasma viral load result from the set point period obtained prior to ART initiation (Table 1). In the serodiscordant couples cohorts 21% (36/168) of HIV-1 seroconverters had schistosomiasis, compared to 23% (46/202) among members of the Mombasa Cohort. The median number of days from collection of the sample used for schistosomiasis testing to the estimated date of HIV-1 acquisition was 14 (interquartile range [IQR], -17 to 46) for the serodiscordant couples cohorts and 74 (IQR, 47 to 127) for the Mombasa Cohort; negative numbers indicate that the participant acquired HIV-1 prior to the date of schistosomiasis testing. The median number of eligible set point viral load results per individual was 4 (IQR, 3 to 6) in the serodiscordant couples cohorts and 3 (IQR, 2 to 5) in the Mombasa Cohort.

In total, there were 1,102 set point plasma viral load results from 288 HIV-1 seroconverters without schistosomiasis (mean = 4.50 $\log_{10}$ copies/ml) and 305 results from 82 seroconverters with schistosomiasis (mean = 4.32 $\log_{10}$ copies/ml). After controlling for age, sex, cohort, and year of HIV-1 acquisition, we found that HIV-1 seroconverters with schistosomiasis had set point plasma viral loads that were a non-significant 0.17 $\log_{10}$ copies/ml lower (95% CI -0.38 to 0.03) than seroconverters without schistosomiasis. To address the possibility that schistosomiasis status may have changed over time, we performed a sensitivity analysis including only samples collected 4-12 months after the estimated date of HIV-1 acquisition and obtained a similar, but statistically significant, result (-0.22 log10 copies/ml, 95% CI -0.43 to -0.01).

Additionally, we performed subanalyses by schistosome species. Compared to individuals without schistosomiasis, individuals infected by *S. mansoni* had statistically significantly lower set point plasma viral loads (-0.34 $\log_{10}$ copies/ml, 95% CI -0.58 to -0.09). In contrast, individuals infected by *S. haematobium* had higher plasma viral loads compared to uninfected individuals (+0.33 $\log_{10}$ copies/ml, 95% CI -0.07 to 0.73), though the result was not statistically significant.

Genital viral load results were only available for the Mombasa Cohort. Out of 202 women with eligible plasma viral load results, 152 had at least one eligible cervical viral load result and 153 had at least one eligible vaginal viral load result. The median number of cervical and vaginal viral load results per individual was 2 (IQR, 1 to 4).

We found that individuals with and without schistosomiasis had similar set point genital viral loads. Individuals with schistosomiasis had cervical set point viral loads that were on average 0.07 $\log_{10}$ copies/swab (95% CI -0.20 to 0.34) higher than uninfected individuals, adjusting for age, year of HIV-1 acquisition, and plasma viral load. Adjusting for the same covariates, individuals with schistosomiasis had vaginal set point viral loads that were 0.11 $\log_{10}$ copies/swab (95% CI -0.17 to 0.39) higher than uninfected individuals. In a sensitivity analysis including only samples collected 4-12 months after the estimated date of HIV-1 acquisition, we still found no evidence that cervical (-0.02 $\log_{10}$ copies/ml, 95% CI -0.35 to 0.30) or vaginal (-0.14 $\log_{10}$ copies/ml, 95% CI -0.49 to 0.21) viral loads were associated with schistosome infection.

Since ova from *S. haematobium* more frequently induce genital damage than ova from *S. mansoni*, potentially leading to inflammation and elevated genital viral load levels, we performed subgroup analyses for each schistosome species. After adjusting for age, year of HIV-1 acquisition, and plasma viral load levels, *S. mansoni* infection was not associated with a statistically significant change in cervical (+0.29 $\log_{10}$ copies/swab, 95% CI -0.04 to 0.62) or vaginal (+0.16 $\log_{10}$ copies/swab, 95% CI -0.19 to 0.52) set point viral loads. In adjusted models, *S. haematobium* infected individuals had lower set point cervical viral loads (-0.59 $\log_{10}$ copies/swab, 95% CI -1.11 to -0.06) but similar set point vaginal viral loads (-0.09 $\log_{10}$ copies/swab, 95% CI -0.65 to 0.46) compared to uninfected individuals.

DISCUSSION

Using data from 370 individuals who HIV-1 seroconverted during prospective follow-up and who contributed 1,407 plasma and 826 genital samples, we found no evidence supporting our hypothesis that schistosomiasis increases HIV-1 plasma or genital viral loads. In fact, *S. mansoni* infection was associated with a statistically significant decline in plasma viral loads, while *S. haematobium* infection was associated with a statistically significant decline in cervical viral loads.

Our findings conflict with a recent study in Tanzania that found *S. mansoni* associated with increased HIV-1 set point viral loads [18]. Interestingly, HIV-1 viral load levels in the two studies were similar among individuals with *S. mansoni* (4.24 vs. 4.4 $\log_{10}$ copies/ml) but differed among schistosome-uninfected individuals (4.50 vs. 3.7 $\log_{10}$ copies/ml). Though set point plasma viral loads vary by population, in other reports from sub-Saharan Africa they typically range from 4.2 to 4.8 $\log_{10}$ copies/ml [94, 100-102]. Thus, the Tanzania study had unusually low plasma viral load levels in their uninfected population, perhaps explained by ART use, which was not monitored in their study population.

Overall, studies that evaluated associations between schistosomiasis and plasma viral load levels in cross-sectional analyses or assessed changes in viral load levels after schistosomiasis treatment have found inconsistent results [18, 87, 103-107]. Since higher set point plasma viral loads are predictive of faster HIV-1 disease progression and mortality [97, 108-112], a positive association between schistosomiasis and HIV-1 would suggest that schistosomiasis plays a role in catalyzing the HIV-1 epidemic. Results presented in this manuscript represent the largest evaluation of this association done to date, and our results do not support this hypothesis. Additionally, our results are consistent with a recent study that found schistosomiasis associated with slower, rather than faster, rates of HIV-1 disease progression [113]. In that manuscript, the authors hypothesized that schistosomiasis may reduce rates of HIV-1 disease progression by increasing numbers of Th17 and T regulatory cells, a marker of immune activation that are suggested to lead to better HIV-1 outcomes. Our results are not fully consistent with this explanation, since upregulation of Th17 and T regulatory cells has been found in individuals with *S. mansoni* or *S. haematobium*, and the lower set point viral loads we observed was specific to *S. mansoni* infection.

Though a positive association between schistosomiasis and genital HIV-1 levels has been hypothesized by others [88, 92, 93], to the best of our knowledge this is the first study to evaluate this association. Since *S. haematobium* more frequently causes genital damage than *S. mansoni* [60, 95], and the cervix is the genital organ most often damaged by schistosome ova [60], we expected to see the higher genital viral loads among cervical samples collected from women infected by S. *haematobium*. Yet, cervical HIV-1 viral loads were actually lower in this population. We lack a possible biological mechanism to explain this result, and acknowledge that we have only eight women infected by *S. haematobium* included in the analysis. This result may have occurred by chance, as we performed multiple statistical tests but did not adjust p-values for multiple comparisons, increasing the probability of identifying a false association.

Utilizing data from four cohort studies, one strength of our analysis is the large sample size of HIV-1 seroconverters included in the plasma set point analysis. Since these individuals were enrolled in ongoing cohort studies, we were able to estimate their date of HIV-1 acquisition with good precision and utilize data collected on potential confounders such as ART use to address possible sources of bias. Our schistosomiasis testing algorithm allowed us to determine the schistosome species causing the infection, enabling us to perform subanalyses by species. Lastly, the availability of both cervical and genital HIV-1 viral load measurements from the Mombasa Cohort allowed us to assess the impact of schistosomiasis on two female genital organs known to experience damage from schistosome ova.

One limitation of our analysis is that schistosomiasis status was measured at a single time point prior to HIV-1 seroconversion. Thus, it is possible that participants' schistosome infection status changed during the set point period. We expect most individuals' schistosomiasis status remained unchanged over the two year period since schistosomiasis acquisition most frequently occurs during childhood [55], individuals in these cohorts were not systematically screened or treated for schistosomiasis, and untreated infections have been shown to persist for up to 40 years [55]. Additionally, to address this concern, we performed a sensitivity analysis restricting sample inclusion to 4-12 months after HIV-1 acquisition, yet we still detected no positive associations between schistosomiasis and HIV-1 set point plasma or genital viral loads. An additional limitation is that the SCAA20 assay lacks the sensitivity to detect very low-burden infections [72].

This could be a modest source of misclassification, but we would expect low burden infections to induce the least amount of genital damage.

In conclusion, we found no evidence that schistosomiasis is associated with increased plasma HIV-1 set point viral loads. Thus, our results do not support the hypothesis that schistosomiasis increases the rate of HIV-1 disease progression. Additionally, we found no evidence that schistosomiasis is associated with increased female genital HIV-1 set point viral loads, providing no evidence to support the hypothesis that schistosome coinfection increases HIV-1 infectivity in women. Though we did not find that schistosomiasis increased plasma or genital HIV-1 viral loads, schistosomiasis induces significant morbidity and mortality, and ongoing support for preventive treatment and schistosomiasis control is needed in many regions of sub-Saharan Africa [84].

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Table 4.1 Characteristics of participants with eligible set point viral load results after HIV-1 seroconversion

	Serodiscordant Couples HIV-1 Seroconverters		Female Sex Workers HIV-1 Seroconverters	
	Schistosome infected (N = 36)	Schistosome uninfected (N = 132)	Schistosome infected (N = 46)	Schistosome uninfected (N = 156)
Age at HIV-1 seroconversion				
16-24	5 (14%)	29 (22%)	10 (22%)	34 (22%)
25-34	16 (44%)	63 (48%)	25 (54%)	85 (54%)
≥35	15 (42%)	49 (30%)	11 (24%)	37 (24%)
Sex				
Female	11 (34%)	70 (53%)	46 (100%)	156 (100%)
Male	21 (66%)	62 (47%)	—	—
Education[2]				
<9 years	28 (78%)	88 (67%)	34 (74%)	98 (63%)
≥9 years	8 (22%)	44 (33%)	12 (26%)	58 (37%)
Married[1]				
Yes	35 (97%)	127 (96%)	28 (61%)	82 (53%)
No	1 (3%)	5 (4%)	18 (39%)	73 (47%)
Enrollment location				
Kenya	11 (31%)	63 (48%)	46 (100%)	156 (100%)
Uganda	25 (69%)	69 (52%)	—	—
Any unprotected sex[3]				
Yes	22 (61%)	95 (72%)	21 (46%)	88 (56%)
No	14 (39%)	37 (28%)	25 (54%)	68 (44%)
Number of sex partners[4]				
≤1	32 (91%)	120 (93%)	38 (83%)	118 (76%)
>1	3 (0%)	9 (7%)	8 (17%)	38 (24%)
Sexually transmitted infections[4]				
Yes	7 (19%)	15 (11%)	10 (22%)	27 (18%)
No	29 (81%)	117 (89%)	35 (78%)	127 (82%)
Serodiscordant couples cohort				
Partners HSV/HIV Transmission Study	21 (58%)	67 (51%)	—	—
Couples Observational Study	1 (3%)	9 (7%)	—	—
Partners PrEP Study	14 (39%)	56 (42%)	—	—
Number of plasma viral load results per participant [Median (IQR)]	4 (3-6)		3 (2-5)	
Number of cervical viral load results per participant [Median (IQR)]*	—	—	2 (1-4)	
Number of vaginal viral load results per participant [Median (IQR)]*	—	—	2 (1-4)	

[1] For the serodiscordant couples cohorts, marital status at the time of study enrollment was assessed. For the FSW cohort, marital status at enrollment was categorized as ever married vs. never married because few participants were married.

[2] Years of education at time of cohort enrollment.

[3] For the serodiscordant couples cohorts, sexual behaviors were assessed over the prior month. Some individuals had missing values for number of sexual partners (n=4). For the FSW cohorts, average sexual behaviors were calculated for each year of cohort follow-up.

[4] For the serodiscordant couples cohorts, sexually transmitted infection testing (trichomoniasis, gonorrhea, and chlamydia) was done at enrollment. For the FSW cohort, sexually transmitted infection testing

(trichomoniasis and gonorrhea) occurred at each study visit and was time-varying, and a few individuals lacked test results (n=3).

Table 4.2 Associations between schistosomiasis and HIV-1 set point plasma viral load

				Bivariate			Adjusted model[1]		
	n obs.	**(N indiv.)**	**Log_{10} copies/ml[2]**	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
No schistosomiasis	1102	(288)	4.50	Ref	—	—	Ref	—	—
Schistosomiasis[3]	305	(82)	4.32	-0.14	-0.34, 0.07	0.184	-0.17	-0.38, 0.03	0.094
	n obs.[4]	**(N indiv.)**	**Log_{10} copies/ml[2]**	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
No schistosomiasis	1102	(288)	4.50	Ref	—	—	Ref	—	—
S. mansoni	205	(53)	4.24	-0.30	-0.55, -0.06	0.015	-0.34	-0.58, -0.09	0.007
S. haematobium	62	(18)	4.61	0.41	0.01, 0.81	0.046	0.33	-0.07, 0.73	0.108
Undetermined species[5]	68	(18)	4.38	-0.05	-0.44, 0.35	0.818	-0.01	-0.40, 0.39	0.971

[1] Adjusted for age, sex, and cohort, plus year of HIV-1 acquisition for the Mombasa Cohort (4-year bands).

[2] The mean log_{10} copies/ml plasma viral loads for individuals with and without schistosomiasis, it does not take into account multiple observations per individual.

[3] Individuals with antischistosomal antibodies (anti-SEA) and schistosome antigens (CAA).

[4] There were 8 individuals (31 observations) who tested positive for both *S. mansoni* and *S. haematobium*.

[5] These individuals had antischistosomal antibodies (anti-SEA) and schistosome antigens (CAA), but tested negative for both *S. mansoni* and *S. haematobium*.

Table 4.3 Associations between schistosomiasis and HIV-1 genital viral loads in the Mombasa Cohort

				Bivariate			Adjusted model[1]		
Cervical HIV-1 viral load	**n obs.**	**(N indiv.)**	**Log_{10} copies/swab[2]**	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
No schistosomiasis	317	(117)	2.87	Ref	—	—	Ref	—	—
Schistosomiasis	86	(35)	2.70	-0.12	-0.49, 0.25	0.522	0.07	-0.20, 0.34	0.623
Vaginal HIV-1 viral load	**n obs.**	**(N indiv.)**	**Log_{10} copies/swab[2]**	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
No schistosomiasis	327	(118)	2.66	Ref	—	—	Ref	—	—
Schistosomiasis	84	(35)	2.62	-0.02	-0.36, 0.31	0.893	0.11	-0.17 , 0.39	0.459

[1] Adjusted for age, year of HIV-1 acquisition (4-year bands), and log_{10} plasma viral loads.

[2] The mean log_{10} copies/swab genital viral loads for individuals with and without schistosomiasis, it does not take into account multiple observations per individual.

Table 4.4 Associations between schistosome species and HIV-1 genital viral loads in the Mombasa Cohort

				Bivariate			Adjusted model[1]		
Cervical HIV-1 viral load[2]	**n obs.**	**(N indiv.)**	**Log_{10} copies/swab[3]**	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
No schistosomiasis	317	(117)	2.87	Ref	—	—	Ref	—	—
S. mansoni	54	(21)	2.72	0.01	-0.47, 0.46	0.983	0.29	-0.04, 0.62	0.089
S. haematobium	18	(8)	2.54	-0.31	-1.04, 0.42	0.404	-0.59	-1.11, -0.06	0.029
Undetermined species[4]	20	(8)	2.80	-0.02	-0.73, 0.70	0.965	0.11	-0.40, 0.62	0.672
Vaginal HIV-1 viral load[2]	**n obs.**	**(N indiv.)**	**Log_{10} copies/swab[3]**	**β**	**95% CI**	***P***	**β**	**95% CI**	***P***
No schistosomiasis	327	(118)	2.66	Ref	—	—	Ref	—	—
S. mansoni	52	(21)	2.58	-0.08	-0.50, 0.34	0.707	0.16	-0.19, 0.52	0.367
S. haematobium	18	(8)	2.83	0.09	-0.56, 0.74	0.718	-0.09	-0.65, 0.46	0.738
Undetermined species[4]	20	(8)	2.73	0.16	-0.47, 0.80	0.615	0.18	-0.36, 0.72	0.507

[1] Adjusted for age, year of HIV-1 acquisition (4-year bands), and log_{10} plasma viral loads.

[2] In both analyses there were 3 individuals (7 observations) who tested positive for both *S. mansoni* and *S. haematobium*.

[3] The mean log_{10} copies/swab genital viral loads for individuals with and without schistosomiasis, it does not take into account multiple observations per individual.

[4] These individuals had antischistosomal antibodies (anti-SEA) and schistosome antigens (CAA), but tested negative for both *S. mansoni* and *S. haematobium*.

Chapter 5. Conclusions

Overview

This dissertation extends our knowledge of trichomoniasis and schistosomiasis, two common HIV-1 coinfections. Results from these analyses help inform the allocation of funding for future public health research and program implementation. The section below briefly summarizes the primary conclusions from my four research aims.

Chapter 2: A cross-sectional analysis of *Trichomonas vaginalis* infection among heterosexual HIV-1 serodiscordant African couples

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Aim 1: **Identify correlates of TV infection within heterosexual couples**

Hypothesis*:* There are modifiable risk factors for trichomoniasis, which have the potential to be addressed by public health interventions.

Conclusions: The strongest predictor of a prevalent *T. vaginalis* infection is having an infected sexual partner. Thus, concurrent treatment of sexual partners is critical to prevent reinfection. Among women, having a circumcised male partner was associated with reduced risk of *T. vaginalis* while bacterial vaginosis was associated with an increased risk, so expanding male circumcision programs and bacterial vaginosis treatment has the potential to reduce the prevalence of trichomoniasis.

Chapter 3: Associations between schistosomiasis and HIV-1 acquisition risk in four prospective cohorts

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Aim 2: **Estimate the association between schistosomiasis and HIV-1 acquisition risk**

Hypothesis*:* Schistosome infection is associated with an increased risk of HIV-1 acquisition.

Conclusions: We found no evidence that *S. mansoni* infection is associated with an increased risk of HIV-1 acquisition and it is unlikely that *S. mansoni* is a major driver of HIV-1 acquisition. *S. haematobium* infection was not associated with a statistically significant increased risk of HIV-1 acquisition, though our point estimate among women in the Mombasa Cohort was elevated (IRR = 1.64). Based on our findings, and the fact that women with *S. haematobium* most often experience genital damage hypothesized to increase HIV-1 susceptibility, any future studies evaluating associations between schistosomiasis and HIV-

1 acquisition should prioritize women infected by *S. haematobium*. Neither species appear to be very strong (RR>3) risk factors for HIV-1, as suggested by previous cross-sectional studies, so any impact of schistosomiasis on the HIV-1 epidemic would be more modest.

Chapter 4: Effects of schistosomiasis on HIV-1 plasma and genital set point viral loads

Aim 3: **Estimate the impact of schistosomiasis on HIV-1 genital viral load in HIV-1 infected women – a marker of HIV-1 transmission risk**

Hypothesis*:* Schistosome coinfection is associated with increased levels of HIV-1 RNA in the genital tract. This association may be stronger among individuals infected by *S. haematobium* than those infected by *S. mansoni.*

Conclusions: We found no evidence that schistosomiasis is associated with increased female genital HIV-1 set point viral loads. Our results do not support the hypothesis that schistosome coinfection increases the risk of HIV-1 transmission.

Aim 4: **Evaluate the association between schistosome coinfection and HIV-1 plasma set-point viral load – a marker for the rate of HIV-1 disease progression**

Hypothesis: Schistosome coinfection is associated with higher HIV-1 RNA set-point in plasma.

Conclusions: We found no evidence that schistosomiasis is associated increased plasma HIV-1 set point viral loads. Thus, our results do not support the hypothesis that schistosomiasis increases the rate of HIV-1 disease progression.

Correlates of *Trichomonas vaginalis*

Our analysis found that the strongest predictor of a prevalent *T. vaginalis* infection is having an infected sexual partner; 23% of women and 61% of men with *T. vaginalis* had a partner with a concurrent infection. Our result highlights the importance of partner treatment to avoid re-infection. This is consistent with the current U.S. Centers for Disease Control and Prevention Guidelines, which recommend presumptive treatment for all current sexual partners to avoid reinfection [3]. Current CDC guidelines do not recommend expedited partner therapy (patient-delivered partner medication); instead, guidelines state that it “might have a role in partner management for trichomoniasis and can be used in states where

permissible by law." This is an area in need of further evaluation. Throughout sub-Saharan Africa, where most sexually transmitted diseases are managed through a syndromic management approach with the etiology never determined, presumptive partner treatment is not routinely implemented. A recent study suggested that point-of-care STI testing, followed by immediate treatment and expedited partner therapy (patient-delivered partner medication) has the potential to reduce re-infection rates in low-income settings [114].

In the near future, wide-spread screening and treatment programs for trichomoniasis are unlikely given the high cost and relatively modest adverse impacts of the disease. Thus, the mostly practical way to impact the population-level prevalence of *T. vaginalis* is by targeting modifiable risk factors. We found that women whose male partners were circumcised had a reduced prevalence of *T. vaginalis* while women with moderate or high Nugent scores for bacterial vaginosis had increased prevalence. Thus, expanding male circumcision programs or bacterial vaginosis treatment have the potential to reduce the prevalence of trichomoniasis. Since bacterial vaginosis is challenging to diagnose, and recurrence after treatment is common, expanded male circumcision, with its other known benefits reducing the transmission risk of other STIs, is the most feasible intervention for reducing the prevalence of *T. vaginalis*.

Associations between schistosomiasis and HIV-1 acquisition

Our analysis provided strong evidence that *S. mansoni* infection is not associated with a large increased risk of HIV-1 acquisition. We found no association in either the serodiscordant couples or female sex worker cohorts. We had good statistical power in both cohorts; the bounds of the 95% confidence intervals for both cohorts excluded an OR/IRR of 1.5. Because existing evidence and the proposed biological mechanisms suggested that an association may be specific to females, the strongest evidence against a strong positive association comes from the female sex worker cohorts (IRR = 0.83, 95% CI 0.58-1.20).

Our findings directly contradict the results of a recent cohort analysis in Tanzania that found a strong association between *S. mansoni* infection and increased HIV-1 acquisition risk among women (aOR = 2.8, 95% CI 1.2-6.6) [18]. There are several possible explanations for this discordant results. First, the

Tanzania analysis may have been conducted in multiple ways to achieve a statistically significant result. In their manuscript, they "defined an individual as schistosome-infected at the time of HIV-1 seroconversion if the dried blood spots collected both before and after HIV-1 seroconversion tested positive for schistosome CAA." This approach is unusual for several reasons: 1) the conventional approach for most exposures is to measure them at the visit prior to an incident event, 2) there is evidence suggesting that damage caused by schistosomiasis persists beyond the period of active infection, so individuals with recently terminated infections may maintain an increased HIV-1 acquisition risk, and 3) if the authors were intending to be conservative, it would have made sense to exclude individuals who transitioned from schistosome-positive to negative (or treat them as a separate group), since this population had mixed schistosomiasis status at the time of HIV-1 acquisition. Performing the analyses in multiple ways increases the probability of finding a false association. However, an alternative explanation for these differing results is that there is a factor present in Tanzania but not among Mombasa FSWs that is modifying the impact of *S. mansoni* on HIV-1 acquisition risk. However, this is unlikely because results from the serodiscordant couples cohorts also did not suggest a strong positive association. Though we had less statistical power among women with *S. mansoni* in the serodiscordant couples cohort, our confidence interval still excluded an odds ratio of 2.8 (aOR = 1.23, 95% CI 0.56-2.68), as observed in the Tanzania study.

Evaluating the association between *S. haematobium* infection and HIV-1 acquisition risk, we had less statistical power than we anticipated; existing data led us to expect that most schistosomiasis in the Mombasa Cohort would be caused by *S. haematobium*, but we instead found that a majority of infections were caused by *S. mansoni* within the cohort (323 vs. 70). In the serodiscordant couples cohorts, only nine women were infected by *S. haematobium*. Our result from the Mombasa Cohort (aIRR = 1.64, 95% CI 0.93-2.87), though not statistically significant, were consistent with a moderate positive association between *S. haematobium* and HIV-1 acquisition risk. We performed many subgroup analyses in this manuscript, which increases the probability of finding a false association and our results did not suggest a dose-response effect. Yet, the strongest epidemiological and biological evidence for an association exists among women with *S. haematobium*, so it is striking that this is the only sub-population with a point estimate of elevated HIV-1 risk.

An important limitation of the subanalyses by species was the performance of the species-specific immunoblots. There are no commercial assays that determine schistosome species from blood samples. Thus, we relied on an in-house assay developed and routinely used by the Parasitic Diseases Branch of the U.S. Centers for Disease Control and Prevention. However, results from the assay suggest that the immunoblots had limited sensitivity and specificity, and this may have meaningfully impacted the results of this analysis. First, we observed an unexpectedly large number of individuals co-infected with both schistosome species. Interestingly, the population of individuals co-infected by both schistosome species contained approximately 25% HIV-1 seroconverters, consistent with a null association given that we selected three controls per case. Thus, if many of the dual-infected individuals were truly negative for *S. haematobium*, the assay misclassification would bias our association between *S. haematobium* and HIV-1 acquisition towards the null. Second, there were an unexpectedly large number of individuals who tested negative for both species, despite being both schistosome antigen and anti-schistosome antibody positive. The population testing negative for both schistosome species contained 36% and 42% HIV-1 seroconverters in the serodiscordant couples and FSW cohorts respectively. If these dual-negative results occurred because of poor sensitivity of the *S. haematobium* immunoblots, and they are truly *S. haematobium* positive, the assay performance may have prevented us from having the statistical power to detect a positive association.

Table 5.1: Species-specific immunoblot results

Serodiscordant Couples Cohorts			
	***S. haematobium* positive N [SC, control][1]**	***S. haematobium* negative N [SC, control][1]**	**Total**
***S. mansoni* positive**	19 [4,15]	115 [27,88]	134 (69%)
***S. mansoni* negative**	17 [6,11]	42 [15,27]	59 (31%)
Total	36 (19%)	157 (81%)	193
Mombasa FSW Cohort			
	***S. haematobium* positive**	***S. haematobium* negative**	**Total**
***S. mansoni* positive**	42 [13,29]	191 [44,147]	233 (81%)
***S. mansoni* negative**	29 [12,17]	26 [11,15]	55 (19%)
Total	71 (25%)	217 (75%)	288

[1] SC, HIV-1 seroconverter (case)

Future directions: schistosomiasis and HIV-1 acquisition

Based on our results, further studies are needed to determine if women with *S. haematobium* infection experience a moderate increase in HIV-1 acquisition risk. Hopefully existing high-risk HIV-1 cohorts can be identified in *S. haematobium* endemic areas, to permit future evaluation of this hypothesis at relatively low cost. Observational studies of the association between schistosomiasis and HIV-1 have the potential to provide strong causal evidence of an association between schistosomiasis and HIV-1 acquisition. Unlike observational studies that evaluate associations between sexually transmitted infections and HIV-1 acquisition, which face a common set of risk factors that make it impossible to completely remove bias caused by confounding, there appear to be few common risk factors for schistosomiasis and HIV-1. This makes sense conceptually, since the mode of transmission for the two diseases differ. We also empirically evaluated a long list of potential confounders of the relationship between schistosomiasis and HIV-1 acquisitions risk and found that none acted as meaningful confounders; factors known to be associated with HIV-1 acquisition risk were not associated with schistosomiasis. An additional way observational studies could provide evidence supporting a causal association would be if future studies find a positive association specific to *S. haematobium* and not *S. mansoni* – *S. mansoni* has the potential to act as a negative control.

If *S. haematobium* is found to increase the risk of HIV-1 acquisition among women in observational studies, designing an intervention to mitigate this risk would be a challenge. First, some results suggest that the damage from schistosome ova may not be reversible. Though inflammation does appear to subside after the infection is resolved [90], damage to the genital mucosa may be permanent [21, 75, 115]. The existing evidence addressing this possibility is surprisingly weak, and future research should evaluate if signs of genital damage such as “sandy patches” on the cervix, or symptoms such as contact bleeding, decline one year after schistosomiasis treatment. The second challenge in designing an intervention is environmental. Though schistosomiasis is treated with a single dose of praziquantel at an estimated cost of US $0.20 per treatment [5], individuals remain at risk of reinfection. Schistosomiasis is contracted through exposure to contaminated freshwater sources such as rivers or lakes [55], and if the source of infection remains, individuals are likely to return to these bodies of water and become re-infected. Thus, continued monitoring and periodic treatment for schistosomiasis is needed.

In light of these challenges, there are several reasons why a prospective study evaluating if *S. haematobium* treatment reduces the risk of HIV-1 should not be pursued in the near future. First, the association between *S. haematobium* and HIV-1 acquisition risk appears to be modest in strength, meaning a very large sample size would be required to detect an association. Second, it would not be ethical to randomized schistosome-infected individuals to receive or not receive treatment. Thus, the study would likely involve a cluster-randomized design, where many communities would need to be randomized to either receive or not receive mass-treatment. Third, the study would either need to include mitigation of environmental exposures (sanitation interventions and/or use the molluscicide niclosamide) or anticipate that many participants would be reinfected, reducing the expected impact of the intervention. Fourth, before an intervention can be designed, researches must understand if the mucosal damage from schistosome ova hypothesized to increase HIV-1 acquisition risk is reversible. If damage is irreversible, treatment would only reduce future genital damage and the effect of the intervention on HIV-1 acquisition risk would be diminished. In this scenario, to completely offset the impact of *S. haematobium* on HIV-1 acquisition risk, participants would need to receive regular treatment starting in early childhood and then be followed for many years until they become sexually active and face a risk of HIV-1 acquisition, which would be impractical.

However, strong observational data suggesting an association between *S. haematobium* and HIV-1 acquisition risk could be persuasive enough to impact future funding decisions. While this data would not be adequate to divert HIV-1 prevention funding to schistosomiasis control, it could be used to advocate for increased basic research into schistosomiasis. For example, currently research is being done to design transgenic snails that are resistant to schistosomiasis [116]. Because snails act as an intermediate host during the schistosomiasis lifecycle [55], treating bodies of water with molluscicide and then repopulating them with schistosome-resistant snail populations could disrupt schistosomiasis transmission for entire communities. Additionally, in many settings there is currently insufficient funding to provide mass-treatment for all eligible high-burden communities [5], and observational data could justify prioritizing areas endemic to *S. haematobium* over *S. mansoni*. Lastly, since schistosomiasis is known to cause morbidity and mortality independent of HIV-1, including kidney failure and bladder cancer [117],

strong evidence from observational studies that *S. haematobium* increases HIV-1 acquisition risk could also be used to advocate for additional funding for schistosomiasis treatment and control.

Associations between schistosomiasis and HIV-1 plasma and genital viral loads

We found no evidence that schistosomiasis infection is associated with increased plasma or genital HIV-1 viral loads. Thus, our results do not support the hypothesis that schistosomiasis coinfection increases the rate of HIV-1 disease progression or the risk of HIV-1 transmission. Since the analysis of schistosomiasis and set point viral loads included both the serodiscordant and female sex worker cohorts, and we did not hypothesize a differing affect by schistosome species, we had strong statistical power to evaluate our primary hypothesis. Though our results were suggestive of a weak negative association among those infected by *S. mansoni*, we are not aware of any biological mechanism that would explain differing results for the two schistosome species. Additionally, schistosomiasis being association with reduced set point viral loads and slower HIV-1 disease progression could not be used to design a public health intervention, since schistosomiasis should be treated to prevent schistosome-induced morbidity and mortality [117].

For the analysis of schistosomiasis infection and genital viral loads, we hypothesized that the positive association may be specific to the sub-population infected by *S. haematobium*. The proposed mechanism for this association was mediated by schistosomal ova damaging the genital mucosa and causing inflammation, and this occurs far more frequently among women infected by *S. haematobium* than women infected by *S. mansoni*. Since the prevalence of *S. haematobium* in the Mombasa Cohort was lower than we anticipated, we had limited statistical power to evaluate this association; cervical and vaginal HIV-1 viral loads were only available for eight *S. haematobium* infected individuals. However, our results suggested a negative, rather than positive, association. We have no proposed mechanism to explain a negative association, and if true, it could not be used to design a public health intervention. We suspect this association may have occurred by chance.

Now that World Health Organization guidelines recommend antiretroviral therapy treatment for all HIV-positive individuals, regardless of their CD4 count, the importance of these types of studies has diminished. Previously, an intervention that slowed HIV-1 disease progression could induce cost-savings by increasing the length of time a HIV-positive individual could wait to initiate antiretroviral therapy, while

an intervention that reduced genital viral loads would reduce transmission risk during the long pre-ART period, but this is no longer the case. Additionally, once an HIV-positive individual initiates antiretroviral therapy, if they remain adherent, their plasma and genital viral loads should become undetectable regardless of the impact of coinfections such as schistosomiasis.

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VITA

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