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

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

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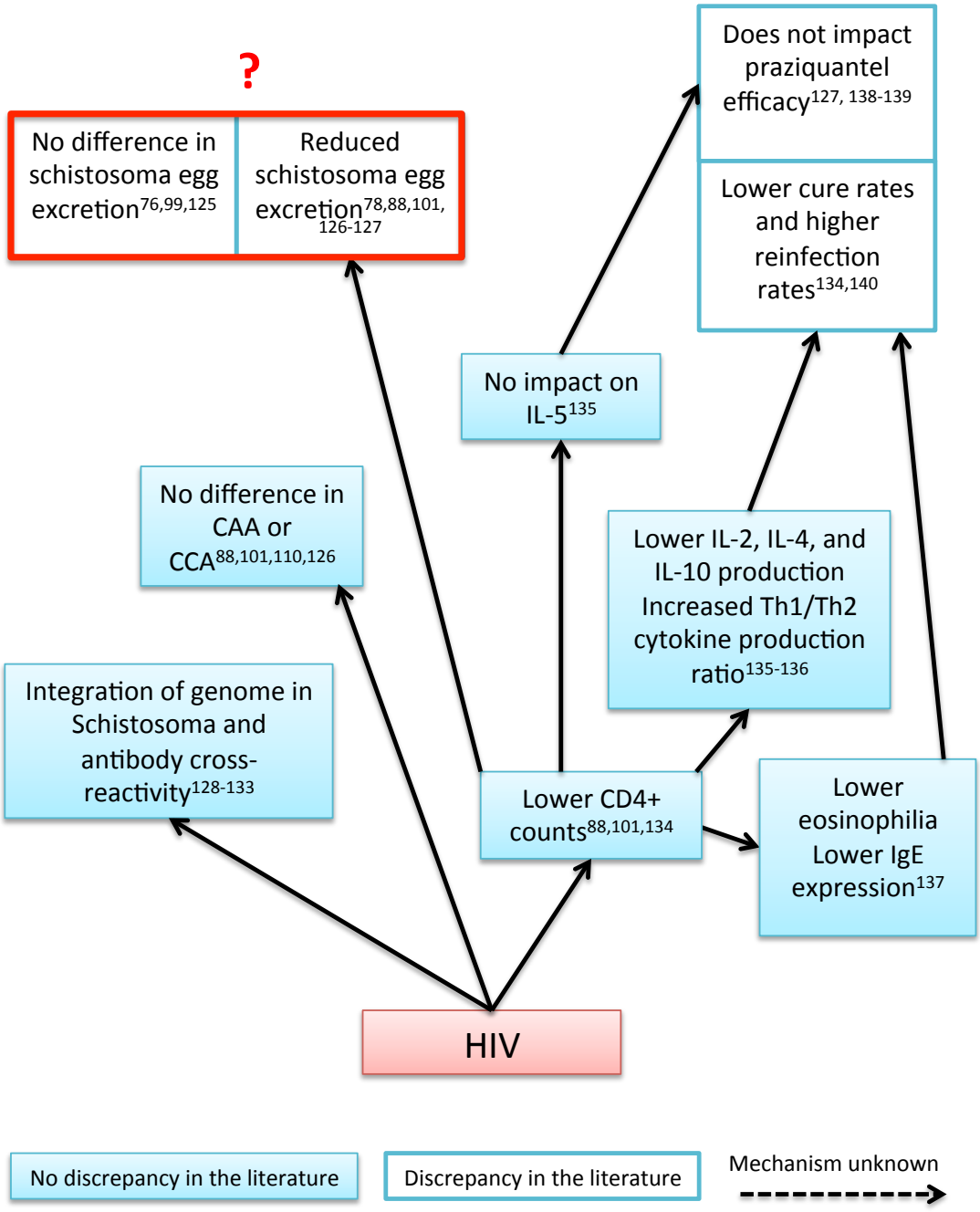
WHERE DOES THIS CHAPTER FIT?



ABSTRACT

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

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

INTRODUCTION

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

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

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

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

METHODS

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

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

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

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

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

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

RESULTS

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

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

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

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

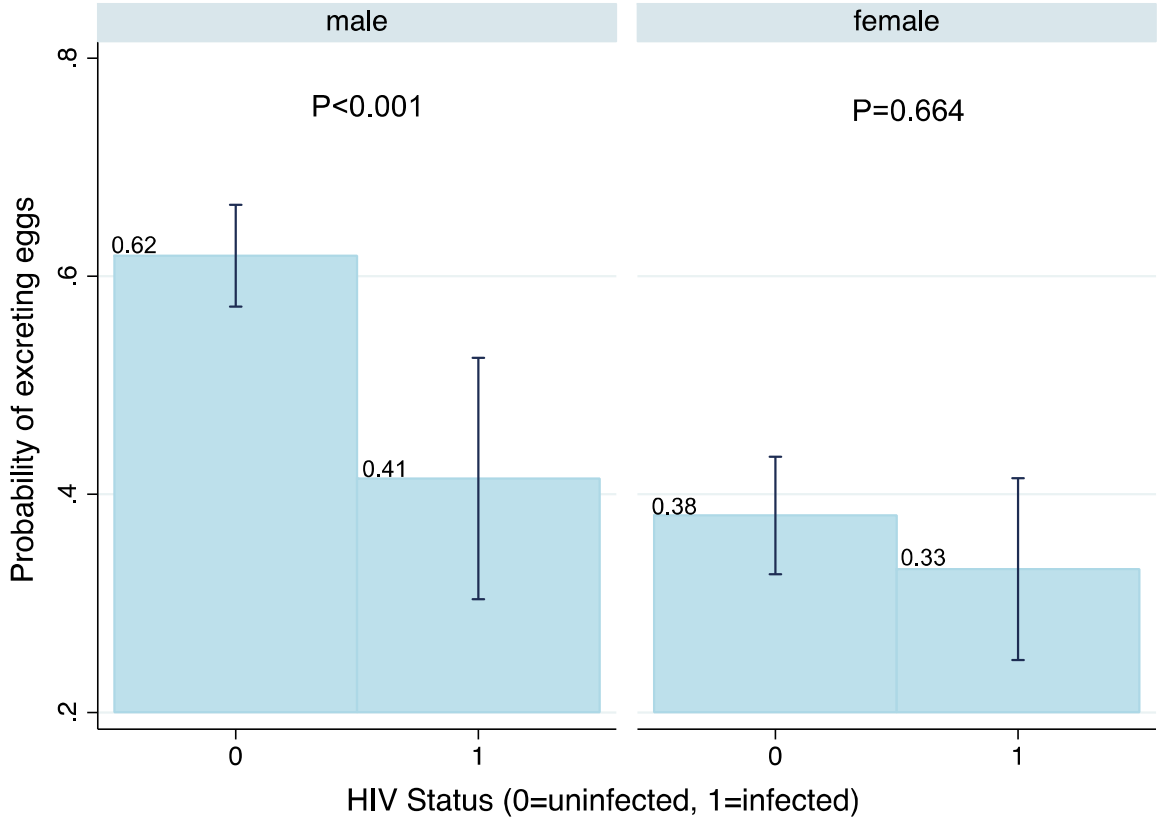
Among the 913 *Schistosoma sp.*-infected individuals, by univariate analysis, HIV infection was associated with lower odds of egg excretion (OR = 0.5 [0.4,0.7], p < 0.001) as was female sex (OR = 0.4 [0.3,0.5], p < 0.001). Past treatment with praziquantel was associated with higher odds of egg excretion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

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

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

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

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



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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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