

# ***Schistosoma mansoni* and HIV acquisition in fishing communities of Lake Victoria, Uganda: a nested case–control study**

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## **Abstract**

**OBJECTIVE** It has been suggested that *Schistosoma mansoni*, which is endemic in African fishing communities, might increase susceptibility to human immunodeficiency virus (HIV) acquisition. If confirmed, this would be of great public health importance in these high HIV-risk communities. This study was undertaken to determine whether *S. mansoni* infection is a risk factor for HIV infection among the fishing communities of Lake Victoria, Uganda. We conducted a matched case–control study, nested within a prospective HIV incidence cohort, including 50 HIV seroconverters (cases) and 150 controls during 2009–2011.

**METHODS** *S. mansoni* infection prior to HIV seroconversion was determined by measuring serum circulating anodic antigen (CAA) in stored serum. HIV testing was carried out using the Determine rapid test and infection confirmed by enzyme-linked immunosorbent assays.

**RESULTS** About 49% of cases and 52% of controls had *S. mansoni* infection prior to HIV seroconversion (or at the time of a similar study visit, for controls): odds ratio, adjusting for ethnicity, religion, marital status, education, occupation, frequency of alcohol consumption in previous 3 months, number of sexual partners while drunk, duration of stay in the community, and history of schistosomiasis treatment in the past 2 years was 1.23 (95% CI 0.3–5.7)  $P = 0.79$ . *S. mansoni* infections were chronic (with little change in status between enrolment and HIV seroconversion), and there was no difference in median CAA concentration between cases and controls.

**CONCLUSIONS** These results do not support the hypothesis that *S. mansoni* infection promotes HIV acquisition.

**keywords** *Schistosoma mansoni*, circulating anodic antigen, HIV, susceptibility, fishing communities, Uganda

## **Introduction**

Fishing communities in Uganda are disproportionately affected by HIV infection with incidence and prevalence four times the national average, or more [1, 2]. These figures are attributed to high-risk sexual behaviour and limited access to health services [1, 2]. *Schistosoma mansoni*, a chronic, immunomodulating helminth infection, highly prevalent in fishing communities, may be an additional

risk factor in these communities [3]. It is estimated that over 16 million Ugandans are at risk of schistosomiasis and more than 4 million infected [4].

*S. haematobium* is a potential risk factor for HIV acquisition, because it directly affects the genital mucosa [5]. *S. mansoni* infection has also been linked to increased risk of acquiring simian–human immunodeficiency virus (SHIV) and HIV in primate and human studies, respectively [3, 6, 7]. Proposed systemic

immunological mechanisms for this include up-regulation of HIV co-receptors and of Th2 T cells in which HIV may replicate more efficiently [3, 8]. If confirmed, this would imply that control of *S. mansoni*, as well as of *S. haematobium*, could contribute to reducing the HIV reservoir among fishing communities and hence spread to the general population.

We conducted a matched case–control study, nested within a prospective HIV incidence study, to determine whether *S. mansoni* infection promotes HIV acquisition in Ugandan fishing communities.

## Methods

The study was conducted among five fishing communities on Lake Victoria, Uganda. This was a matched case–control study nested within a prospective HIV incidence cohort of 1000 individuals (males and females, aged 13–49 years) recruited in 2009, and followed for 18 months [2].

Within the prospective cohort, data on risk factors for HIV acquisition, and serum samples for HIV testing and storage, were obtained at enrolment and at six-monthly follow-up intervals. HIV testing was carried out using Determine rapid tests (Abbott Laboratories, Chicago, IL, USA), confirmed by enzyme-linked immunosorbent assays (ELISA; Vironostika HIV Uni-Form II plus 0 microelisa system, Biomerieux, Boxtel, Netherlands and Murex HIV-1.2.0, Murex, Biotech Limited, Dartford, UK), with Western blot (Calypse Biomedical Corporation, Rockville, Maryland, USA) if ELISA results were indeterminate. During follow-up, 59 individuals became HIV-1 positive [2].

All cases available and willing to participate were selected. Fifty HIV seroconverters were included as cases, with 150 controls selected from those who remained HIV negative. For every case, three controls matched on age, sex and community of residence were randomly selected by computer using StataSE12 (1985–2011 StataCorp, College Station, TX, USA). Controls that were not willing to participate or could not be traced after more than three attempts were replaced.

At the case–control study visit, participants were interviewed regarding schistosomiasis risk factors. Stored serum was tested for *Schistosoma* circulating anodic antigen (CAA) at cohort enrolment, 6, 12 and 18 months using CAA-specific up-converting phosphor technology-based lateral flow assay strips (UPT-LF) [9, 10], and a CAA value  $\geq 32$  pg/ml was considered positive. Although CAA cannot distinguish *S. mansoni* from *S. haematobium*, the latter has not been found on the Ugandan shores of Lake Victoria [11], so a positive result was taken as indicative of *S. mansoni* infection. For cases, CAA serostatus was taken as the result for the last sample before

HIV seroconversion. For controls, CAA serostatus was taken as the result for the sample corresponding to the last visit before HIV seroconversion for the matched case.

Stool samples were also collected during the case–control study visit (after the end of cohort follow-up), for three consecutive days, to test for *S. mansoni* infection. Two laboratories participated. Microscopy was performed using the Kato–Katz method in one location, formal–ether concentration in the other location. Intensity of infection was defined based on WHO criteria as light ( $<100$  eggs per gram (epg), moderate (100–399 epg) and heavy ( $\geq 400$  epg).

Participants diagnosed with HIV infection (during follow-up) were referred to The AIDS Support Organization (TASO) and Entebbe Hospital for care and treatment according to the Uganda Ministry of Health guidelines. Those diagnosed with *S. mansoni* infection (after the end of follow-up) were treated with single-dose praziquantel, 40 mg/kg. Participants diagnosed with other helminth infections were treated with a single dose of albendazole 400 mg.

Data were double-entered using MS Access 2003 (1992–2003 Microsoft Corporation, Redmond, Washington, USA) and analysed using StataSE12.

The outcome was HIV seroconversion during follow-up. The aim was to determine whether *S. mansoni* infection (assessed by serum CAA) was associated with the outcome (HIV seroconversion during follow-up). Associations between stool microscopy results and serum CAA were also investigated.

Baseline age, sex and community of residence were matching factors. Matched conditional logistic regression was used to investigate univariable associations between HIV seroconversion and other demographic factors (marital status, education status, occupation, religion and behavioural risk factors; being away from home for at least two nights in the previous month; frequency of alcohol use; and number of sexual partners while under the influence of alcohol or drugs during the previous 3 months) and to investigate risk factors for *S. mansoni* infection (duration of stay in fishing community and history of treatment for schistosomiasis in the preceding 2 years). Covariates considered *a priori* to be potential confounders (marital status, religion and ethnicity) and those associated with *S. mansoni* at  $P \leq 0.1$  were assessed for confounding by adding them individually to the multivariable model and tested for goodness of fit using log likelihood ratio test. If the *S. mansoni*–HIV effect measure changed by 10% or more, the covariate was retained in the final model.

The study was approved by Uganda Virus Research Institute Science and Ethics Committee (FWA number

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00001354) and the Uganda National Council of Science and Technology (FWA number 00001293). Participants aged 18 years and above were enrolled after giving written informed consent. Those aged 13–17 years were enrolled after written assent, with consent from their parents or guardians.

## Results

### Socio-demographic characteristics

Characteristics of the cohort have been described [2]. Among the 200 participants enrolled into this nested study, median age was 25 years (IQR 22–30 years). The highest proportion was males (56%), Roman Catholics (41%), married (63%), with incomplete primary-level education (41%) and worked in fishing-related jobs (66%).

### Schistosomiasis

The majority of participants (76%) had not received *S. mansoni* (praziquantel) treatment in the previous 2 years. Determination of *S. mansoni* infection status was based on serum CAA.

Half (51%) had *S. mansoni* infection at cohort enrolment, 48%, 52% and 52% at 6, 12 and 18 months, respectively. There was 96% agreement between the CAA status at enrolment and CAA status at the pre-seroconversion (or equivalent control) time point ( $\kappa=0.91$ ,  $P < 0.001$ ). Compared to the stool results, serum CAA had a sensitivity of 84%, specificity 92% and a positive predictive value of 94%. Intensity of *S. mansoni* infection was assessed in 79 samples examined by the Kato–Katz method. Of those infected, 61% had light, 29% moderate and 10% heavy infections.

*S. mansoni* prevalence was highest in the 25–29 years age group, [66%,  $P < 0.001$ ], higher among males than females [75% *vs.* 21%,  $P < 0.001$ ] and increased with declining education level [ $P = 0.035$ ]. Almost all participants who were engaged in fishing had *S. mansoni* infection [54/58, 93%;  $P < 0.001$  compared to other occupational groups]. Being away from home frequently [ $P = 0.009$ ] and long-term residence in the fishing community [ $P = 0.012$ ] were associated with *S. mansoni* infection.

### Association between *S. mansoni* infection and HIV acquisition

There was no difference between cases who acquired HIV infection, and controls who had not, in *S. mansoni*

infection status based on CAA results at the preceding visit: 49% of cases and 52% of controls had *S. mansoni* infection [OR 0.83 (95% CI 0.39–1.77)  $P = 0.63$ ]. The result was similar after adjusting for ethnicity, religion, marital status, education, occupation, frequency of alcohol consumption in previous 3 months, number of sexual partners while drunk, duration of stay in the community and history of schistosomiasis treatment (praziquantel) in the past 2 years [aOR 1.23 (95% CI 0.3–5.7)  $P = 0.79$ ]. There was also no difference in median CAA concentration between cases and controls: 2906 and 6530 pg/ml, respectively ( $P = 0.16$ ).

Factors associated with HIV acquisition in the case–control study were similar to those previously reported for the whole cohort: occupation, religion, frequency of alcohol consumption (2); Table 1. In addition, not having taken praziquantel treatment for *Schistosomiasis* in the last 2 years was associated with HIV acquisition [OR 8.25 (95% CI 1.3–51.1)  $P = 0.03$ ].

### Discussion and conclusion

We found that *S. mansoni* infection was not associated with HIV acquisition among adults in Lake Victoria fishing villages. As opposed to *S. haematobium*, *S. mansoni* is unlikely to cause genital lesions. This result therefore suggests that systemic immune modulation by *S. mansoni* does not significantly increase susceptibility to HIV acquisition.

By contrast, an experiment in macaques showed increased susceptibility to a simian–human immunodeficiency virus (SHIV) infection in *S. mansoni*-infected animals [6]. However, the macaque schistosome infections were recent at the time of SHIV exposure (7 weeks), egg counts were equivalent to moderate to heavy human infections, and the virus was administered rectally, encountering tissues directly affected by *S. mansoni* egg-induced lesions. By contrast, our human participants had chronic *S. mansoni* infections (few had recently been treated, and status had not changed between enrolment and the pre-seroconversion visit), many had light infections, and HIV infection would usually have occurred via the genital mucosa. Indeed, in a second macaque study, *S. mansoni* infection did not increase susceptibility when SHIV was administered intravenously, implying that route of infection was critical [7].

Cross-sectional studies have provided conflicting evidence regarding HIV–*S. mansoni* associations. A survey focussing on women on the Tanzanian shores of Lake Victoria using serum CAA to determine *Schistosoma* infection found a positive association with HIV infection,

**Table 1** Multivariable conditional regression analysis of factors associated with HIV infection

Variable	Unadjusted		Adjusted		P-value
	OR	95% CI	OR	95% CI	
Serum CAA	0.8	0.4–1.8	1.23	0.3–5.7	0.79
Marital status					
Premarital	1 Ref		1 Ref		0.5
Married Monogamous	1.00	0.4–2.6	1.14	0.19–6.76	
Married Polygamous	0.55	0.2–2.0	0.36	0.33–4.02	
Divorced/Widowed	1.36	0.4–4.4	0.52	0.55–4.86	
Education Status					
Post-primary	1 Ref		1 Ref		0.94
Completed Primary	1.42	0.6–3.6	0.64	0.1–3.9	
Incomplete Primary	1.20	0.5–2.8	0.94	0.2–5.2	
None	1.61	0.5–5.3	1.00	0.1–8.5	
Occupation					
Fishing	1 Ref		1 Ref		<0.005
Fishing related	1.13	0.4–3.1	13.54	1.8–101.3	
Bar/Hotel/restaurant	2.92	0.6–15.1	41.53	1.6–1071.3	
Business/trade	0.49	0.1–2.6	0.32	0.0–6.3	
Farming	1.57	0.4–6.0	8.84	0.7–110.6	
Other jobs	2.22	0.5–10.5	4.00	0.3–52.5	
Not earning	1.85	–	4.78	–	
Religion					
Catholic	1 Ref		1 Ref		0.018
Anglican	5.56	2.3–13.5	7.98	1.8–35.4	
Muslim	1.65	0.7–3.9	2.73	0.7–10.9	
Other	1.70	0.4–7.2	9.06	0.7–112.3	
Ethnicity					
Non-Muganda	1 Ref		1 Ref		0.32
Muganda	0.8	0.4–1.6	0.50	0.1–2.0	
Alcohol use					
Never	1 Ref		1 Ref		0.013
Rarely	1.92	0.8–4.8	9.21	1.4–61.8	
Regularly	3.49	1.6–7.8	6.85	1.3–37.4	
Duration of stay					
5–45 years	1 Ref		1 Ref		<0.005
<5 years	3.77	1.6–8.6	7.56	1.5–37.6	
Bilharzia treatment in last 2 years					
Yes	1 Ref		1 Ref		0.034
No	2.49	1.0–6.3	8.25	1.3–51.1	
Sexual partners while drunk(pre-infection)					
0	1 Ref		1 Ref		0.98
1	1.96	0.8–4.7	0.67	0.1–3.2	
≥ 2	1.58	0.4–6.0	0.48	0.0–7.0	

and higher CAA levels among HIV-positive women [12]. However, the number of HIV-infected women was small (21 of 345) so confidence intervals were wide, the prevalence of HIV infection was approximately 4.5 times lower than in the Ugandan fishing villages, and the catchment area extended to 25 km from the lake, so that a wide array of potential confounding factors in terms of environment and lifestyle may have come into play. A

low prevalence of *S. haematobium* (3%) might also have contributed.

The cross-sectional design of the Tanzanian study means that the sequence in which the infections occurred is unknown. An earlier study showed no positive association between *S. mansoni* and HIV infection and highlighted differences in occupation and origin of members of the population affected by the two infections [13].

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Given the lack of association we observed between *S. mansoni* infection and HIV acquisition, we were surprised to observe a strong association between HIV infection and lack of praziquantel treatment in the last 2 years. It is difficult to draw robust conclusions from self-reported behaviour. We presume this may be a marker for poor health-seeking behaviour, rather than for a biological process. Participants were not examined or treated for *S. mansoni* by the research team during the cohort study.

Strengths of our study were prospective sample collection, recruitment restricted to a relatively uniform (fishing village) community, and matching and adjustment for potential confounders. The CAA assay allowed assessment of *S. mansoni* infection status at enrolment and immediately prior to HIV seroconversion, demonstrating chronicity of infection, and avoided potential concerns about differential egg excretion among HIV-positive and HIV-negative people [14]. Although it can be difficult to assess causality in case-control studies, the prospective design of our cohort did allow us to assess *Schistosomiasis* status prior to HIV acquisition – an important improvement over studies with a cross-sectional design.

While there are many potential benefits of *S. mansoni* control in heavily infected communities, our results suggest that an impact on HIV transmission is not one of them.

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### References

1. Kiwanuka N, Ssetaala A, Mpendo J *et al.* High HIV-1 prevalence, risk behaviours, and willingness to participate in HIV vaccine trials in fishing communities on Lake Victoria, Uganda. *J Int AIDS Soc* 2013; **16**: 18621.
2. Seeley J, Nakyingi-Miuro J, Kamali A *et al.* High HIV incidence and socio-behavioral risk patterns in fishing communities on the shores of Lake Victoria, Uganda. *Sex Transm Dis* 2012; **39**: 433–439.
3. Mazigo HD, Nuwaha F, Wilson S *et al.* Epidemiology and interactions of Human Immunodeficiency Virus – 1 and *Schistosoma mansoni* in sub-Saharan Africa. *Infect Dis Poverty* 2013, **2**: 2.
4. Kabatereine NB, Standley CJ, Sousa-Figueiredo JC *et al.* Integrated prevalence mapping of schistosomiasis, soil-transmitted helminthiasis and malaria in lakeside and island communities in Lake Victoria, Uganda. *Parasit Vectors* 2011; **4**: 232.
5. Kjetland EF, Hegertun IE, Baay MF, Onsrud M, Ndhlovu PD, Taylor M. Genital schistosomiasis and its unacknowledged role on HIV transmission in the STD intervention studies. *Int J STD AIDS* 2014; **25**: 705–715.
6. Chenine AL, Shai-Kobiler E, Steele LN *et al.* Acute *Schistosoma mansoni* infection increases susceptibility to systemic SHIV clade C infection in rhesus macaques after mucosal virus exposure. *PLoS Negl Trop Dis* 2008; **2**: e265.
7. Siddappa NB, Hemashettar G, Shanmuganathan V *et al.* *Schistosoma mansoni* enhances host susceptibility to mucosal but not intravenous challenge by R5 Clade C SHIV. *PLoS Negl Trop Dis* 2011; **5**: e1270.
8. Secor WE. Interactions between schistosomiasis and infection with HIV-1. *Parasite Immunol* 2006; **28**: 597–603.
9. Corstjens PL, De Dood CJ, Kornelis D *et al.* Tools for diagnosis, monitoring and screening of *Schistosoma* infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology* 2014; **141**: 1841–1855.
10. Corstjens PL, van Lieshout L, Zuiderwijk M *et al.* Up-converting phosphor technology-based lateral flow assay for detection of *Schistosoma* circulating anodic antigen in serum. *J Clin Microbiol* 2008; **46**: 171–176.
11. Emmanuel IO, Ekkehard D. Epidemiology, of bilharzias (schistosomiasis) in Uganda from 1902 until 2005. *Afr Health Sci* 2008; **8**: 239–243.
12. Downs JA, van Dam GJ, Changalucha JM *et al.* Association of Schistosomiasis and HIV infection in Tanzania. *Am J Trop Med Hyg* 2012; **87**: 868–873.
13. Fontanet AL, Woldemichael T, Sahlu T *et al.* Epidemiology of HIV and *Schistosoma mansoni* infections among sugar-

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- estate residents in Ethiopia. *Ann Trop Med Parasitol* 2000; **94**: 145–155.
14. Karanja DM, Colley DG, Nahlen BL, Ouma JH, Secor WE. Studies on schistosomiasis in western Kenya: I. Evidence for immune-facilitated excretion of schistosome eggs from patients with *Schistosoma mansoni* and human immunodeficiency virus coinfections. *Am J Trop Med Hyg* 1997; **56**: 515–521.

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