The cervicovaginal environment and HIV incidence in Zambian women with female genital schistosomiasis

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Thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy

of the
University of London
July 16, 2021

Department of Clinical Research

Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

To the BILHIV community workers, Livingstone-based Zambart staff, and study participants – your strength, dedication, commitment, and tenacity have touched me in unforgettable ways.

"Every time you love just a little
Take one step closer, solving a riddle
It echoes all over the world"

-Dar Williams, Echoes

Declaration of Own Work

I, Amy Sorensen Sturt, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature

Date: July 16, 2021

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Abstract

Background:

In 2019, an estimated 56 million women were living with female genital schistosomiasis (FGS), a neglected tropical disease that results when eggs from the waterborne parasite *Schistosoma (S.) haematobium* are deposited in reproductive tissues. FGS has been associated with important sexual and reproductive health consequences, including ectopic pregnancy, infertility, and human immunodeficiency virus (HIV-1) acquisition. Inflammation in the female genital tract is likewise associated with HIV-1 acquisition and "non-optimal" cervicovaginal microbiota have also been associated with adverse reproductive consequences, including pelvic inflammatory disease, infections of pregnancy and the post-partum period and HIV-1 acquisition. The cervicovaginal environment of women with FGS has not been fully described. The overall aim of this PhD thesis was to describe the cervicovaginal microbiota (including STI) and the cervicovaginal immune environment, focusing on cytokines and chemokines, in Zambian women with and without FGS and to explore the association of FGS with HIV-1 incidence.

Methods:

This PhD work was nested within the bilharzia and HIV (BILHIV) study, which recruited women aged 18-31, sexually active, and not pregnant from the HPTN 071 (PopART) Population Cohort, from which longitudinal information on HIV-1 infection status was available. Women enrolled in the BILHIV study (n=603) were assessed for FGS through self-collected genital swabs and clinic-collected CVL (n=527). For this PhD work, all BILHIV participants with FGS (n=30) and all participants with probable FGS (n=25) were selected, and three FGS negative participants were selected for every FGS and probable FGS participant using a random number

generator, frequency matched by age to participants with FGS. Among selected participants, cervicovaginal microbiota and STI were quantified using PCR and the associations of presence, median (IQR), and log concentration mean with FGS status were assessed. The concentrations of 17 soluble cytokines and chemokines were quantified in cervicovaginal lavage (CVL) by a multiplex bead-based immunoassay to evaluate the association between FGS and concentration of cervicovaginal cytokines and chemokines. To explore the association of FGS with HIV-1 incidence, the rate of HIV-1 seroconversion was assessed among women who were HIV-1 negative at enrolment in the HPTN 071 (PopART) Population Cohort (n=492) and associations with FGS were evaluated with exact Poisson regression.

Results:

Of 603 women enrolled in BILHIV, 5.0% (30/603) had FGS, defined as PCR-detected *Schistosoma* DNA in any of three genital specimens (cervical swab, vaginal swab, or CVL). The prevalence of schistosome infection in the study population was 5.5% (33/603) by urine microscopy and 15.1% (91/601) by urine CAA. The presence and concentration of cervicovaginal species did not differ between participants with or without FGS. However, a higher proportion of participants with FGS had *T. vaginalis* compared to FGS negative women (p=0.08). An exploratory analysis suggested an association of *T. vaginalis* presence with FGS among women with ≥2 *Schistosoma* PCR positive genital specimens (50.0%, 8/16) compared with FGS negative (21.5% 34/158, p=0.01).

There was no difference in the concentrations of cytokines or chemokines between participants with and without FGS. After adjusting for potential confounders, an exploratory analysis of

women with ≥2 genital specimens with detectable *Schistosoma* DNA (n=15) showed a higher Th2 (IL-4, IL-5, and IL-13) and pro-inflammatory (IL-15) expression pattern in comparison to FGS negative women. After adjusting for multiple testing, the association between IL-4 ($p=0.037$) and IL-5 ($p<0.001$) and FGS were unlikely to be due to chance.

Incident HIV-1 infections were observed in 4.1% (20/492) participants. Women with FGS were twice as likely to seroconvert as women without FGS but with no statistical evidence for a difference (aRR 2.16, 95%CI [0.21–12.30], p=0.33). Exploratory analysis suggested a point estimate consistent with increased risk of HIV-1 acquisition among women with ≥2 positive genital PCR specimens (RR 6.02, [0.58–34.96]), p=0.13), without statistical evidence of a difference after adjusting for potential confounders.

Conclusion: FGS may alter the cervicovaginal environment, particularly in high burden infections. There are higher HIV-1 seroconversion rates in women with FGS, although power to detect an association was limited in this analysis. The burden of disease should be assessed and correlated with outcomes in participants with FGS. Ideally, a longitudinal study would evaluate the interaction between FGS, HIV-1, and the cervicovaginal environment to provide additional depth to these findings.

Acknowledgements

This PhD thesis is a global collaboration and has brought together people, institutions, countries, and funders. Without support from The Wellcome Trust, the BILHIV study (and thus this PhD) would not have been possible. I am likewise grateful to the trustees of the Dowager Countess Eleanor Peel Trust and Grace McComsey for purchasing the MAGPIX assay kits. My thanks also go to LSHTM for the Doctoral Travel Scholarship.

I cannot write adequate expressions of praise and gratitude for my supervisors Amaya Bustinduy, Emily Webb, and Suzanna Francis, who each devoted time, expertise, and heart in guiding me along this journey. I stand in awe of your capabilities, commitments, and dedication. Despite competing demands, you found time and enthusiasm to share your vision, direction, and skills. You inspire me!

I would like to thank my upgrading examiners Rashida Ferrand and Julius Hafalla for refining this proposal to showcase the portions that "sing". Additionally, I owe deepest thanks to my advisory committee, Dr. Helena Helmby and Professor Richard Hayes. Professor Hayes, thank you for letting me be a part HPTN 071 (PopART) and for thoughtful comments on each manuscript. Thank you to Dr. Helena Helmby for igniting my interest in FGS, ultimately directing me to Amaya, brainstorming the MAGPIX experiment, and always being willing to talk parasite immunology. Thank you to STEPH/SME/ASME teachers and tutors for helping me learn statistics and STATA. Thank you to Piet Cools for teaching me DNA extraction and PCR techniques at the University of Ghent. Special thanks go to Catriona Patterson at LSHTM for teaching me bead-based assays and for practical support during the technical journey. Thank you to the Great Western Railway for my mobile office during many years of commuting into London.

I am grateful to the BILHIV study participants for sharing their time and personal experiences, without you this work would not have been possible! To the hard-working BILHIV field workers (Mwiingana, Mervis, Ruth, Ethel, Judy) our crackerjack BILHIV team supervisor Namakau Chola, fabulous Zambia-based project manager Comfort Rutty-Phiri, reliable drivers George Chaila and Hillary Sipulila, excellent lab technician Tobias Mweene, and supportive site director Clement Mwakamui – you have all inspired me with your boundless enthusiasm, energy, and commitment and I am better for having worked alongside you. To Maina Mudenda and Joyce Mapani, our midwives who so warmly welcomed BILHIV patients into their cervical cancer clinic – thank you! Deepest thanks are reserved for Dr. Isaiah Hansingo for his unwavering support of BILHIV and the sub-studies.

I would like to thank the army of carers who loved Eden, Asher, and Axel while I was investing heart in this PhD project. I would like to thank my family: my parents for always believing in me; Eden, Asher, and Axel for unconditional love and for giving me, hands-down, the three best reasons to close the laptop; and particularly Colin for bringing delight, joy, grace, and companionship to all aspects of existence – but also for his bedrock support, for patience with me as I am, and for his vision of who I can be.

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Glossary of definitions, acronyms and abbreviations

4PL	4 parameter logistic regression
5PL	5 parameter logistic regression
16S rRNA	16S ribosomal RNA
Active infection	a schistosome infection characterized by live schistosome worms producing circulating anodic antigen or with detectable eggs in excreta, or both
aHR	adjusted hazard ratio
aOR	adjusted odds ratio
BILHIV	the bilharzia and HIV study – a cross-sectional study in Zambia to compare the performance of vaginal and cervical self-sampling with provider performed cervicovaginal lavage
BV	bacterial vaginosis
CAA	circulating anodic antigen
CCA	circulating cathodic antigen
CCD	charged coupled device
Ct	cycle threshold
CV	coefficient of variation
CVL	cervicovaginal lavage
DNA	deoxyribonucleic acid
DRC	Democratic Republic of the Congo (DRC)
ELISA	enzyme-linked immunosorbent assay
EFK	Eyrun Floerecke Kjetland
FGS	female genital schistosomiasis
HIV-1	human immunodeficiency virus-1
HR	hazard ratio
HPV	human papillomavirus
HPTN 071 (PopART)	HIV-1 prevention trial network 017 population ART trial – a trial to measure the impact of an HIV combination prevention package, including universal HIV-1 test and treat
HSV-2	human simplex virus-2
IL	interleukin
IFN-γ	interferon-gamma
IQR	interquartile range
LED	light emitting diode
LF	lateral flow
LLOQ	lower limit of quantification
LUMC	Leiden University Medical Center
LSHTM	London School of Hygiene and Tropical Medicine
M1 macrophage	classically activated macrophage
M2 macrophage	alternatively activated macrophage
MCP-1	monocyte chemoattractant protein-1
MFI	mean fluorescent intensity
MIP-1α	macrophage inflammatory protein-1α

MIP-1β	macrophage inflammatory protein-1β
Mixed infections	studies evaluating both *S. haematobium* and *S. mansoni* by microscopy
ODK	Open Data Kit
OR	odds ratio
QC	quality control
QCBT	quantitative crushed biopsy technique
Pap	Papanicolaou
PC	Population Cohort
PCR	polymerase chain reaction
PFU	plaque forming unit
PSA	prostate specific antigen
RPR	rapid plasma reagin
RNA	ribonucleic acid
QC	quality control
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
S. haematobium antibody status	studies using serology to evaluate for past schistosome infection
STI	sexually transmitted infection
STIs	sexually transmitted infections
TCA	trichloroacetic acid
Th1	t-helper type 1
Th2	t-helper type 2
Tissue FGS	studies using a tissue diagnosis as the FGS diagnostic reference standard
TNF-α	tumour necrosis factor-α
UCP	up-converting reporter particle
UNAIDS	United Nations Programme on HIV/AIDS
Unspecified schistosome infection	studies evaluating circulating cathodic antigen (CCA) or CAA in isolation
Urogenital schistosomiasis	urinary *S. haematobium* infection (in the absence of evaluation for genital involvement)
Visual FGS	studies using the presence of any of the four recognized FGS cervicovaginal manifestations as the diagnostic reference standard
WHO	World Health Organization

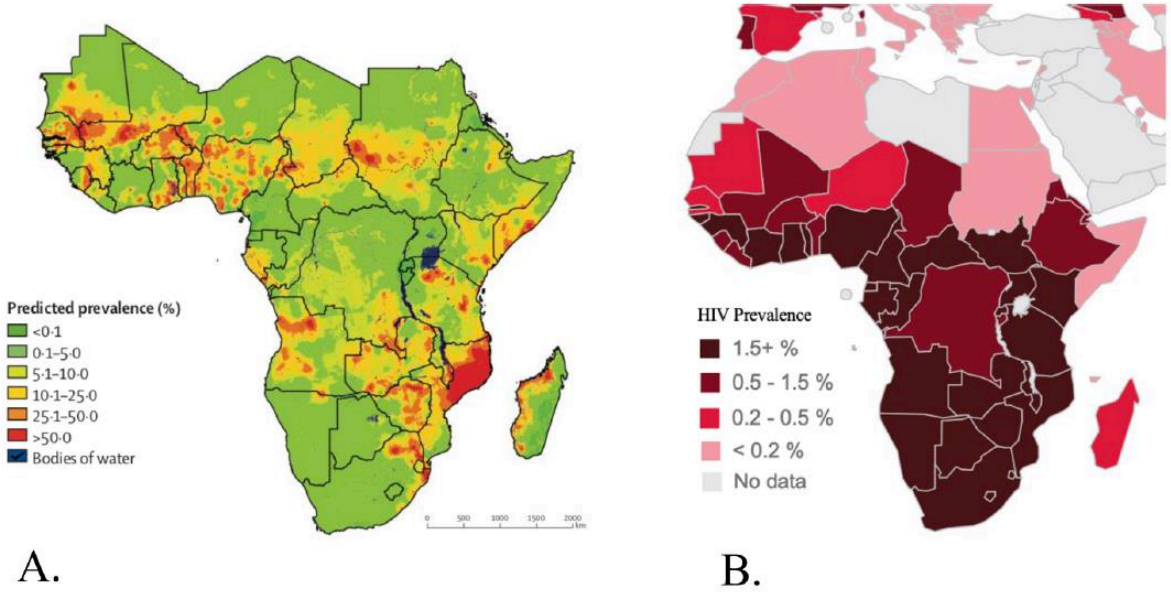
Chapter 1 – Introduction

Schistosomiasis is a Neglected Tropical Disease, caused when human hosts come into contact with fresh water contaminated by *Schistosoma* larvae [1, 2]. The cercarial larvae penetrate intact skin and become schistosomula which mate and mature within the host bloodstream [1, 3]. Adult *Schistosoma (S.) haematobium* blood flukes often inhabit the vesicular venous plexus, where the venous anastomoses with the female reproductive tract allows parasite eggs access to the urogenital organs [4]. Instead of exiting the human host through the urinary tract to continue the life-cycle, some *S. haematobium* eggs become trapped in tissue of the female genital tract, giving rise to female genital schistosomiasis (FGS). Eggs that return to fresh-water in human excreta hatch to release miracidia. When these miracidia find a suitable snail intermediate host, asexual reproduction commences, ultimately releasing cercariae and completing the life-cycle[1].

Globally, 779 million people are estimated to be at risk for *Schistosoma* infection [5]. Six species of *Schistosoma* can infect humans: *Schistosoma mansoni*, *Schistosoma haematobium*, *Schistosoma japonicum, Schistosoma mekongi, Schistosoma intercalatum,* and *S. guineensis [1, 5]*. This PhD thesis will focus on *S. haematobium*, which primarily occurs in the Middle East and sub-Saharan Africa. *S. mansoni, S. intercalatum, S. guineensis and S. mansoni* can also be found in sub-Saharan Africa and *S. mansoni* is also present in the Americas [1, 5]. Two species are localised to Asia, with *S. mekongi* along the Mekong River in Laos and Cambodia and *S. japonicum* primarily in China and the Philippines [1, 5]. The global burden of schistosomiasis is estimated at 1.7 -4.5 million disability-adjusted life years with morbidities in children including anaemia and stunted growth [5]. Thus, the World Health Organization (WHO) recommends preventive chemotherapy to prevent morbidity due to schistosome infection. WHO estimates that 290.8 million people required preventive chemotherapy for schistosomiasis in 2018, 90% of whom live in Africa [6]. Modelling data suggests that 82 million African women are living with *Schistosoma* infections [7], and urinary and genital manifestations are both commonly seen in women. Varied estimates of the population prevalence of FGS amongst reproductive age women are available from cross-sectional studies, ranging from 32 to 75% (32% (n=263), Tanzania [8]; 46% (n=24), Ghana [9]; 65% (n=51), Malawi [10]; 75% (n=61), Niger [11]). Thus, the approximately 10,000 FGS cases reported in the published literature are likely to be an underestimate of the true FGS burden in sub-Saharan Africa [12].

In 2019, UNAIDS estimates that 38 million people worldwide are living with HIV-1 infection, 54.0% (20.7 million) of whom live in Eastern and Southern Africa [13]. In this region, areas of *Schistosoma* endemicity overlap with areas of high HIV-1 prevalence (Figure 1.1) [14]. In 1994, Feldmeier et al, published an editorial suggesting that FGS might be a risk factor for HIV-1 acquisition, hypothesizing biological plausibility for a causal association and ushering in an era of FGS research and advocacy [15]. Subsequent modelling studies from sub-Saharan Africa suggested that each *S. haematobium* infection per 100 individuals was associated with a 2.9% increase in HIV-1 prevalence [16]. Likewise, cross-sectional studies have shown an association between urogenital schistosomiasis and HIV-1 [17], though there is evidence both for an against this association [18, 19].

Figure 1.1 – Overlapping distributions of schistosomiasis and HIV-1 prevalence



Adapted from [14]

1.1 Urogenital schistosomiasis

Autopsy studies in *S. haematobium* endemic areas suggest that parasite eggs are primarily found in the bladder (urinary schistosomiasis) but can also commonly be found in the female genital tract with a higher proportions of eggs in the cervix and vagina compared with the Fallopian tubes and ovaries [20, 21]. Urinary schistosomiasis and

FGS can overlap, with up to 61% of women with biopsy-proven FGS also having urinary *S. haematobium* infection [22, 23]. Recognizing the propensity for urinary and genital schistosomiasis to co-exist, the WHO convened an informal working group in 2009 to propose terminology and discuss the potential association between FGS and human immunodeficiency virus-1 (HIV-1) [24]. FGS was defined as "the presence of ova in the female reproductive organs or a characteristic clinical pathology" [24]. In analogy to the French "bilharziose urogenitale", a new term for urinary *S. haematobium* infection, "urogenital schistosomiasis", was proposed [24]. In the literature, "urogenital schistosomiasis" is commonly used to refer to women with urinary *S. haematobium* infection.

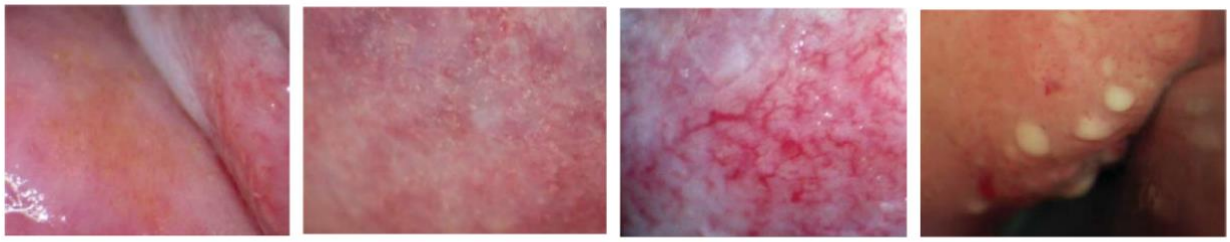
1.2 Diagnostics - *Schistosoma* and female genital schistosomiasis

Urinary *S. haematobium* infection can be diagnosed with either of two "reference standard" diagnostics, urine microscopy or circulating anodic antigen (CAA). Currently, however, a "reference standard" diagnostic does not exist for genital schistosomiasis. Thus, an FGS evaluation is often based on a combination of diagnostic tests including CAA, urine microscopy, colposcopy, tissue-based diagnostics [biopsy, Papanicolaou (pap) smear, wet prep], and polymerase chain reaction [PCR]) [17, 25, 26].

1.2.1 Colposcopy and visual identification of FGS-related cervicovaginal manifestations

Four primary cervicovaginal clinical manifestations have been associated with FGS: grainy sandy patches, homogenous yellow sandy patches, rubbery papules, and abnormal blood vessels (Figure 1.2) [27, 28]. While abnormal blood vessels are included as an FGS criterion in the WHO Pocket Atlas (Figure 1.2), they are less specific than sandy patches (grainy and homogeneous) and rubbery papules, primarily due to the association of abnormal cervical blood vessels with cervical dysplasia [28]. However, sandy patches (grainy and homogeneous) and rubbery papules also lack specificity. Homogeneous yellow sandy patches have been associated with sexually transmitted infections (STIs) (human papillomavirus [HPV], herpes simplex virus type 2 [HSV-2], and *Chlamydia (C.) trachomatis* [26]) and rubbery papules can appear similar to non-infectious findings such as Nabothian cysts [28].

Figure 1.2 – Visual findings suggestive of female genital schistosomiasis



Homogeneous Yellow Sandy Patch | Grainy Sandy Patch | Abnormal Vessels | Rubbery Papules

adapted from [14, 27]

The WHO definition for FGS includes both the presence of parasite eggs in genital tissue as well as "a characteristic clinical pathology" [24] which can be identified by a trained medical professional and requires the insertion of a vaginal speculum and magnification of the vagina and cervix. For clinical purposes, either criterion would suffice to provide a patient with praziquantel treatment. However, there may be challenges in research settings when cervicovaginal clinical manifestations alone are used to diagnose FGS in the absence of tissue diagnostics or molecular methods. For example, the interpretation of FGS cervicovaginal manifestations may not be reproducible across reviewers (Bustinduy, A., unpublished data) and the imperfect specificity of FGS cervicovaginal manifestations presents the possibility for confounding by STIs and cervical dysplasia [26, 28].

1.2.2 Polymerase chain reaction for the detection of Schistosoma *DNA*

Early FGS studies employed tissue-based diagnostic techniques (biopsy, wet-prep, pap smear) [10, 23] to identify *S. haematobium* eggs in genital tissue. However, FGS researchers have advocated for limiting the routine use cervicovaginal biopsy in FGS research due to the theoretical risk of HIV-1 acquisition at the biopsy site [29]. PCR detection of *Schistosoma* deoxyribonucleic acid (DNA) has been identified as a semi-quantitative and less invasive means to identify the presence of viable parasite eggs, compared with biopsy. The sensitivity of *Schistosoma* PCR is imperfect, as illustrated by a Zimbabwean study that evaluated the performance of CVL PCR [29]. However, this may be related to the use of visually identified FGS as a reference standard. In the Zimbabwean study, PCR for *Schistosoma* DNA (n=83) was performed on CVL and compared against a composite FGS endpoint defined by the results of tissue biopsy (n=6), wet mount (n=25), pap smear (n=74) and FGS-related cervicovaginal manifestations (n=83) as the reference standard with a sensitivity of

53% and specificity of 70% [29]. The BILHIV study, a cross-sectional study in Zambia reported that vaginal or cervical swab *Schistosoma* PCR had a sensitivity of 80% compared with a composite endpoint of any genital PCR positive specimen (CVL, vaginal swab or cervical swab) [25].

1.2.3 Circulating Anodic Antigen

Active infection with the genus *Schistosoma* can be detected by the presence of CAA, a polysaccharide antigen regurgitated from the parasite's gut into the host's bloodstream [30, 31]. CAA is partially filtered by the kidneys, making it possible to measure CAA in both serum and urine specimens [30, 32]. The concentration of CAA in urine is lower compared with blood, requiring a concentration step prior to urine specimen processing [30]. Parasite antigens decline after parasite death, making them useful markers of active schistosome infection, as well as response to praziquantel treatment [30, 33-35]. CAA has higher sensitivity than urine microscopy, and this is particularly evident in areas of low background *Schistosoma* prevalence, since CAA is more likely than urine microscopy to detect low intensity infections [36]. However, while useful in detecting active infection, detectable CAA levels do not confirm genital infection status.

1.2.4 Urine Microscopy

S. haematobium eggs are excreted in the urine and traditional microscopy can be used to identify the terminal-spined morphology diagnostic of active infection [37]. There are challenges intrinsic in the use of traditional microscopy including variable egg excretion based on host circadian rhythm and volume status [38], as well as a diminished ability to detect true positive *S. haematobium* infections in low-endemic settings [39]. The intensity of *S. haematobium* infection can be categorized into light (1-49 eggs) and heavy (>=50 eggs) by the number of eggs excreted per 10mL urine [39, 40]. Additional infection intensity sub-classes have also been proposed: ultra-light (1-5 eggs) and light (11-49 eggs) [39]. Urine microscopy may be appropriate for moderate and heavy *S. haematobium* infections, but this technique is less likely to successfully detect light [36] or ultra-light infections [39]. The diminished sensitivity of urine microscopy is particularly important in communities targeted for elimination, especially after receiving repeated preventive chemotherapy and treatment with praziquantel [41]. Similar to antigen testing, the presence of urinary schistosomiasis does not confirm genital infection status.

1.2.5 Haematuria

Haematuria is a defining sign of *S. haematobium* infection and can be microscopic or macroscopic [3, 39]. Macroscopic haematuria can be assessed visually in epidemiologic surveys or through standardized questionnaires [37]. Macroscopic haematuria often declines in a population after preventive chemotherapy or other treatment with praziquantel, and thus may be challenging to follow longitudinally [42]. Chemical reagent strips that detect microhaematuria are an inexpensive and rapid indirect method of detecting urinary *S. haematobium* morbidity [42]. Compared to urine filtration, testing with chemical reagent strips is less expensive, requires less technical expertise, and results are less impacted by parasite circadian rhythms [43]. However, reagent strips do not provide estimates of *Schistosoma* infection intensity or information regarding genital involvement. Like many diagnostic tests, the performance characteristics of chemical reagent strips vary by *S. haematobium* prevalence, with lower sensitivity reported in low-intensity infection and previously treated populations [39, 42]. As a low-cost alternative for estimating *S. haematobium* infection, the use of chemical reagent strips remains useful in population surveys and for determining community and individual level prevalence in high prevalence and pre-preventive chemotherapy settings, but may need to be followed by more sensitive testing in low-prevalence and low-intensity settings [44].

1.3 Literature review of *Schistosoma* infection categories and the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence

In February 2020, prior to the submission of Research Paper 1, I performed a PubMed search with the following search terms “Schistosomiasis” OR “*Schistosoma*” OR “female urogenital schistosomiasis” OR “genital or urogenital schistosomiasis” AND “HIV or human immunodeficiency virus”. The references of relevant articles were also used to identify additional resources. Thus, Research Paper 1 primarily describes literature evaluating the association between FGS and HIV-1 prevalence [14].

The following section of this PhD thesis includes a review of the literature regarding schistosome infection and the cervicovaginal cytokines and chemokines, the cervicovaginal microbiota (including STIs), HIV-1 prevalence and HIV-1 incidence. In April 2021, I extended the findings from Research Paper 1 by performing additional searches to also include manuscripts that reported

schistosomiasis infection with HIV-1 incidence, cervicovaginal cytokines and chemokines, or the cervicovaginal microbiota (including STI) as outcomes. For the cervicovaginal cytokines and chemokines, I searched using the following search terms: ("Schistosomiasis" OR "*Schistosoma*") AND ("cervicovag* or vagina or vaginal or cervix or cervical or genital") AND ("inflamm* or cytokine or chemokine or IL"). For the cervicovaginal microbiota, I searched using the following search terms ("Schistosomiasis" OR " *Schistosoma*") AND ("cervicovag* or vagina or vaginal or cervix or cervical or genital") AND ("microbiota or microbiome or lactob* or bacteri* or anaerob* or atopobium or vaginae or BV or bacterial vaginosis or sexually transmitted infection [STI] or (sexual* transmit*)"). Additionally, the search for Research Paper 1 was repeated from February 2020.

Given the varying definitions of FGS and urogenital schistosomiasis often employed in the schistosomiasis literature, and the frequent use of composite endpoints, the following section will use specific *Schistosoma* case definitions to contextualize the current literature regarding schistosome infection and HIV-1 incidence, HIV-1 prevalence, cervicovaginal cytokines and chemokines, as well as the cervicovaginal microbiota and STIs. This PhD thesis will focus primarily on women with *S. haematobium* infection. However, to provide additional context, a short discussion of *S. mansoni* related literature is included.

For this PhD thesis literature review, six mutually exclusive groups of differing *Schistosoma* infection diagnostic strategies were created: 1) *Schistosoma* antibody positivity, 2) unspecified, mixed, or *S. mansoni* infection (all schistosomiasis studies with the above specified outcomes that do not focus on *S. haematobium* or FGS), 3) urogenital schistosomiasis (in *S. haematobium* endemic areas or with primary evaluation for *S. haematobium*), 4) visual FGS, 5) tissue FGS (including composite endpoints), and 6) PCR-defined FGS. If a study utilized multiple diagnostic techniques and a reference standard was not identified, the study was grouped according to the diagnostic that was used to identify active infection (e.g. studies using both antibody status and CAA are grouped with the CAA studies [45] and studies using both urine microscopy and antibody status are categorised with urogenital schistosomiasis [19]). Identified manuscripts were included if they enrolled participants with above *Schistosoma* case definitions in combination with one of the following outcomes: cervicovaginal cytokines and chemokines, the cervicovaginal microbiota/STIs, HIV-1 prevalence or HIV-1 incidence.

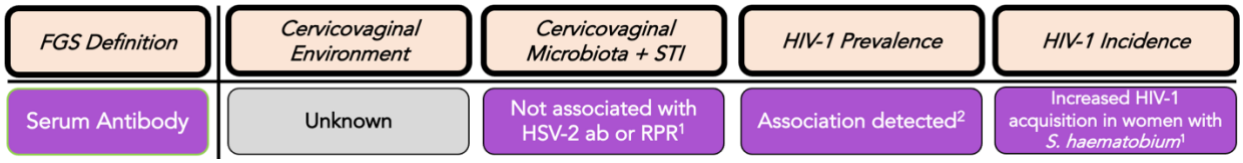
1.3.1 Schistosoma *antibody positivity*

Since antibody status cannot differentiate active versus prior infection, this *Schistosoma* diagnostic method is not used commonly in endemic areas [46]. I identified two studies which used *S. haematobium* antibody status to evaluate an association with any of the following outcomes: the cervicovaginal environment, the cervicovaginal microbiota/STIs, HIV-1 prevalence or HIV-1 incidence (Figure 1.3) [47, 48]. Both studies showed an association between schistosomiasis antibody status and HIV-1 infection, one with prevalent HIV-1 [48] and one with incident HIV-1 [47].

In the first study, a cohort of heterosexual HIV-1 serodiscordant couples in Lusaka, Zambia was followed longitudinally, evaluating baseline HIV-1 prevalence, HIV-1 incidence and STIs [47]. HIV-1 seroconverters and a random sample of non-seroconverters were evaluated in a nested case-control study and plasma and serum samples were tested for schistosome soluble worm antigen preparation by ELISA with species-specific antigens used to differentiate *S. mansoni* and *S. haematobium* by immunoblot. In a sub-group of couples with a HIV-1 positive male partner and a HIV-1 negative female partner, women with *S. haematobium* specific antibodies had an increased risk (adjusted hazard ratio [aHR] 1.4, p=0.034) of HIV-1 acquisition [47]. Baseline *Schistosoma* antibody status was not associated with serum HSV-2 antibody or baseline rapid plasma reagin (RPR) results [47]. There was no evidence of an association between HIV-1 acquisition in women with *S. mansoni* infection (aHR 1.33 (0.93 – 1.91, p=0.12) [47].

In the second, transient elastography was performed to evaluate the prevalence and correlates of liver fibrosis in Uganda, comparing HIV-1 positive and negative participants [48]. *Schistosoma* infection was determined by soluble egg antigen ELISA status. There was strong evidence that a higher proportion of participants living with HIV-1 had *Schistosoma* antibodies (14%, 71/500) than those who were HIV-1 negative (9%, 46/500) (p=0.014) (Table 1.1a) [48].

Figure 1.3 – "*Schistosoma* antibody" positivity and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence.



Abbreviations: Ab – antibody, HSV-2 – herpes simplex virus-2, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, STI – sexually transmitted infection

1.3.2 Unspecified schistosome, mixed schistosome, or S. mansoni *infection*

This PhD thesis focuses primarily on *S. haematobium*. However, a brief review of the literature regarding mixed, unspecified, and *S. mansoni* infection is relevant for context. Thus, I included studies in this category which do not primarily report *S. haematobium* infection. To be included, some proportion of the population required a diagnosis of active schistosome infection (defined by either positive microscopy or circulating parasite antigens, or both) in the absence of evaluation for genital schistosomiasis. Parasite antigens are not species specific [49], and thus studies evaluating circulating cathodic antigen (CCA) or CAA in isolation are defined in this PhD thesis as "unspecified schistosome infection"(Table 1.1b). Studies evaluating both *S. haematobium* and *S. mansoni* by microscopy are defined as "mixed" infections (Table 1.1c), even if individual participants were confirmed to have only one of the two species. I identified thirteen studies in this "unspecified/mixed/*S. mansoni"* category: ten evaluating HIV-1 endpoints (Table 1.1b, 1.1c, 1.1d) [45, 50-58], one evaluating the cervicovaginal microbiota as an endpoint (Table 1.1e [59], one evaluating the cervicovaginal immune environment as an endpoint (Table 1.1f, Figure 1.4) [60] and one evaluating STIs as an endpoint [61] (Table 1.1g).

Four studies of "unspecified infection" showed disparate results with regards to HIV-1 endpoints (Table 1.1b). Two studies from fishing communities in Tanzania [58] and Uganda [55] enrolled both women and men and did not show an association between schistosome infection and either HIV-1 acquisition [55] or prevalent HIV-1 [58] despite analysis by participant sex in the Tanzanian study [58]. Two additional Tanzanian studies showed an association between schistosome infection and HIV-1, but only among female participants [52, 57]. The first was a cross-sectional study in Tanzania which found that schistosome infection was associated with prevalent HIV-1 infection

(OR 3.9 (1.3 – 12.0), p=0.015) [52]. The second was a Tanzanian cohort that assessed schistosome infection status at baseline and evaluated HIV-1 acquisition over time, showing that women with a positive CAA had increased odds of HIV-1 seroconversion compared to CAA negative women (adjusted odds ratio [aOR] 2.8 [1.2 – 6.6, p=0.019), however this association was not seen among men [57] (Figure 1.4).

Three studies of "mixed" *S. haematobium* and *S. mansoni* infections did not show an association between schistosome infection and prevalent HIV-1 [45, 51, 56] (Table 1.1c, Figure 1.4). One was a case-control study nested within a longitudinal cohort that used both *Schistosoma* species specific antibodies and CAA to evaluate active infection but did not show an association between active schistosome infection and HIV-1 seroconversion despite subgroup analyses by sex, schistosome species, and infection intensity [45]. Cross-sectional studies from Tanzania and Zimbabwe evaluated the association between prevalent HIV-1 and schistosomiasis and found no evidence of association [51, 56]. However, the Tanzanian study was conducted only in men [56]. Three additional studies of *S. mansoni* infection (using primarily stool microscopy [50, 53, 54], with CAA [54] or CCA [50] done only in subgroups) enrolling both men and women did not show an association between schistosome infection and prevalent HIV-1 [50, 53, 54], however only one of these studies performed sub-analysis by participant sex [54] (Table 1.1d).

Of the thirteen included studies of participants with "unspecified/mixed/*S. mansoni"* infection, one manuscript evaluated the cervicovaginal microbiota as a study endpoint (Table 1.1e) [59]. In this small longitudinal Tanzanian study, where women were evaluated at baseline and after treatment with praziquantel, women with *S. haematobium* infection (n=17, serum CAA positive and urine microscopy positive) were compared with *S. haematobium* negative women (n=27, CAA and urine microscopy negative) and women with *S. mansoni* infection (n=39, serum CAA positive and stool microscopy positive) were compared with *S. mansoni* negative women (n=52, CAA and stool microscopy negative) (Table 1e) [59]. Alpha diversity is a summary statistic which describes within sample diversity for one participant, while beta diversity measures similarity between samples. Comparing *S. haematobium* positive and *S. haematobium* negative women at baseline, alpha diversity (1.83 vs. 1.36, p= 0.39) and beta diversity (p = 0.60) were not different between groups. However, in participants with high-intensity *S. haematobium* infections there was strong evidence of higher alpha diversity than in *S. haematobium* negative participants (2.43 vs. 1.37, p=0.016) [59].

Comparing *S. mansoni* positive and *S. mansoni* negative women at baseline, alpha diversity (2.03 vs. 1.88, p= 0.32) and beta diversity (p= 0.515) were not different between groups [59].

A small longitudinal study in Tanzania evaluated cervicovaginal cytokines as a study endpoint [60]. In this study, women with concordant serum CAA and microscopy results (with both urine microscopy for *S. haematobium* and stool for *S. mansoni* to evaluate for single species infection*),* were enrolled and the concentration of cervicovaginal lavage cytokines was compared in *S. haematobium* positive (n=18) and *S. haematobium* negative (n=39) women and between *S. mansoni* positive (n=11) and *S. mansoni* negative (n=29) women (Table 1.1f) [60]. Women with urinary *S. haematobium* infection had lower concentrations of IL-15 than women without *S. haematobium* infection (p=0.013) [60]. No difference in cervicovaginal cytokines was detected in women with and without *S. mansoni* infection. Of note, in this study among a subset of cervical swabs available for testing, there were no significant differences in the prevalence of *C. trachomatis*, *N. gonorrhoeae*, or *T. vaginalis* between those with and without schistosome infection [60].

None of the evaluated studies reported an association between "unspecified/mixed/*S. mansoni"* infection and STIs. Of the thirteen studies of "unspecified/mixed/*S. mansoni"* infection, three evaluated STIs as study endpoints [58, 60, 61]. One study evaluating fishing communities in Tanzania did not detect an association between STI and "unspecified/mixed/*S. mansoni"* infection [58]. Results of a study of Tanzanian women are discussed in the previous paragraph [60]. A study of Ugandan women evaluated *C. trachomatis*, *N. gonorrhoeae*, or *T. vaginalis* infection and did not find that detection of these STIs as a group was associated with "unspecified/mixed/*S. mansoni"* infection [61]. However, there was weak evidence that *C. trachomatis* prevalence was associated with schistosomiasis (p=0.06) (Table 1.1g).

Although genital infections occur in *S. mansoni*, egg deposition is primarily in the colon and small intestine [62]. Eggs transiting through the intestinal walls undoubtedly cause disruption in the gut mucosal barrier, which could allow increased access to HIV-1 virions to submucosal target cells [63]. Additionally, *S. mansoni* infection has been associated with the presence of rectal pro-inflammatory cytokines, independent of the native microbiota [64]. The inflammatory environment may also increase barrier permeability [65]. Indeed, macaque research suggests that primates with

systemic *S. mansoni* infection acquired simian-HIV infection after rectal inoculation at doses that were sub-infectious in *S. mansoni* uninfected hosts [66].

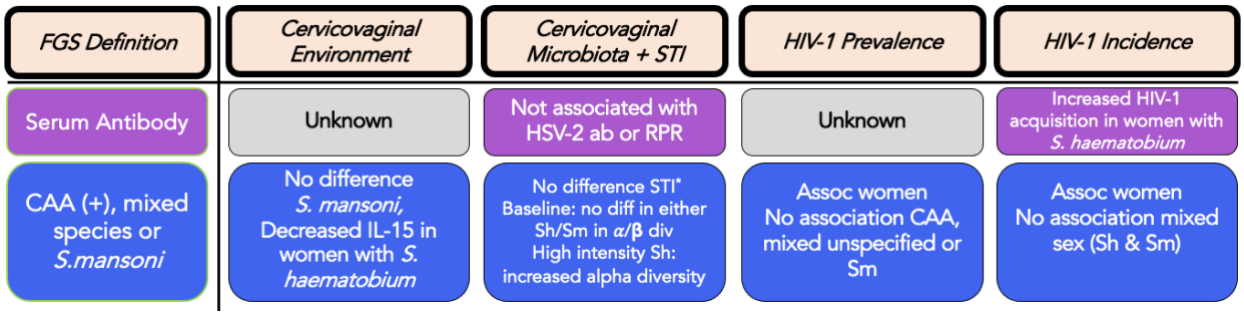
While many hypotheses exist, the exact mechanism of the potential association between *S. mansoni* infection and HIV-1 has not yet been defined. Some mechanistic hypotheses for the association between *S. mansoni* and HIV-1 include *Schistosoma*-related direct mucosal effects, effects on HIV-1 target cells, and parasite modulation of the local and systemic immune response [63]. The literature describes HIV-1 susceptibility in women with schistosome infection more commonly than in men. A Tanzanian series of women with biopsy-proven FGS described that nearly half (47.4%, 9/19) of participants with biopsy-proven FGS had clinically visible cervical lesions, arguing for a role for direct mucosal effects in *S. mansoni* infection [67].

Additionally, the organs most commonly involved in male genital schistosomiasis are the prostate and seminal vesicles [68], internal structures not exposed during sexual contact. Additionally, the HIV-1 vulnerability in *S. mansoni* infection seen in women, but not men [56, 57], may suggest a role of the cervicovaginal mucosa in HIV-1 vulnerability associated with this species. In addition to the impact on gut inflammation and barrier dysfunction discussed above, an additional hypothesis is that *S. mansoni* may be involved in activation of the common mucosal immune system [69] with subsequent $CD4^+$ T cell recruitment to the cervix via integrin $\alpha4\beta7$ [70]. This integrin does not home to the foreskin, providing a potential mechanism for HIV-1 vulnerability based on sex [63]. Additional mechanisms supporting HIV-1 vulnerability include *S. mansoni* effects on target cells and HIV-1 coreceptors. For example, Th17 cells are preferential targets for HIV-1 infection [71] and *S. mansoni* infected Ugandan men were found to have increased Th17 cells in the blood compared to uninfected men, suggesting a potential role of increased HIV-1 target cells in *S. mansoni* associated HIV-1 susceptibility [72]. *S. mansoni* may also increase HIV-1 susceptibility through an observed association with higher levels of the HIV-1 co-receptors CCR5 and CXCR4 on $CD4^+$ T-cells [73]. Additional mechanisms to consider would be *S. mansoni* mediated effects on cervicovaginal gene expression, microbiota and the immune environment. The literature review of "unspecified/mixed/*S. mansoni"* infection identified two studies evaluating the cervicovaginal microbiota [59] and the immune environment [60] as study endpoints. While small and hypothesis generating, these studies did not detect a difference between *S. mansoni* infection and baseline alpha

or beta diversity or cytokine concentrations [59, 60]. Additionally, cervical gene expression has not been shown to differ between *S. mansoni* infected and uninfected women [60].

In summary, in the thirteen studies that evaluated "mixed/unspecified/*S. mansoni"* infection but did not specifically assess genital involvement, I identified ten studies that evaluated HIV-1 as a study endpoint (75.0%, 9/12) (Table 1.1b, 1.1c, 1.1d) [50-58]. Overall, only a minority of studies (16.7%, 2/12) show an association between schistosome infection and HIV-1, and if present, the association was observed in studies evaluating women or sub-groups of women [52].

Figure 1.4 – "Unspecified schistosome, mixed schistosome, or *S. mansoni* infection" and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence



Abbreviations: Ab – antibody, Assoc – association, CAA – circulating anodic antigen, diff – difference, HSV-2 – herpes simplex virus-2, IL – interleukin, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, Sh – *S. haematobium*, Sm – *S. mansoni,* STI – sexually transmitted infection

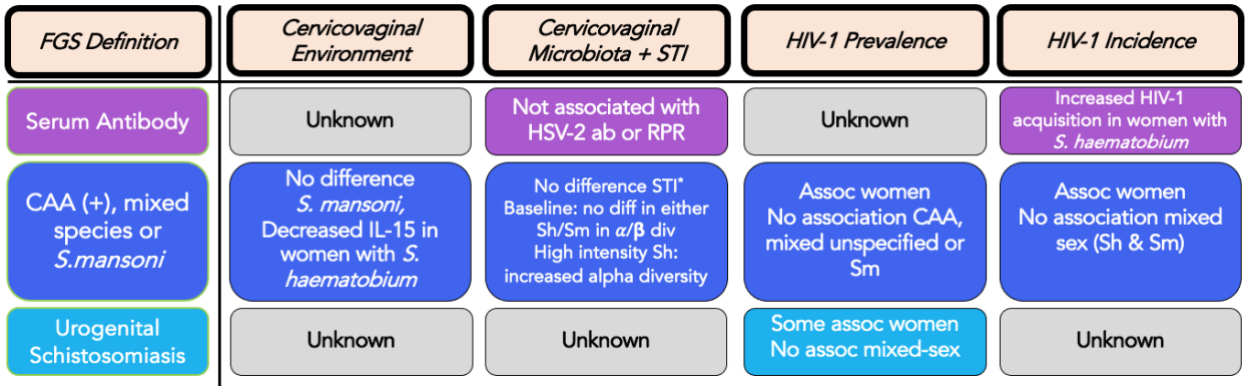
1.3.3 Urogenital schistosomiasis

Included manuscripts for "urogenital schistosomiasis", primarily evaluated urinary *S. haematobium*, in the absence of evaluation for genital involvement [18]. Three studies evaluated either prevalent HIV-1 or STI endpoints (Table 1.1h, Table 1.1i, Figure 1.5). The studies evaluating HIV-1 as an endpoint showed mixed results [18, 19]. One study in Zimbabwean women (n=544) showed some evidence of an association between "urogenital schistosomiasis" and HIV-1, with women with "urogenital schistosomiasis" having a higher HIV-1 prevalence (33.3%, 72/216) compared with women without "urogenital schistosomiasis" (25.6% 84/328, p=0.053) [18]. However, in a sub-group analysis by age (<35 years), there was evidence of an association between "urogenital

schistosomiasis" and HIV-1, with women above 35 years of age and urinary *S. haematobium* having a higher HIV-1 prevalence (37.5%, 33/88) compared to *S. haematobium* negative women (16.8%, 30/179, p<0.001) [18]. Additionally, a study of men and women in the Democratic Republic of the Congo (DRC) (n=895) did not show evidence of a difference in prevalent HIV-1 between participants with (3.2%, 5/156) and without (5.9%, 44/739) urinary *S. haematobium* infection (p=0.18) [19]. An analysis to compare outcomes by participant sex was not performed [19].

The study evaluating STI as an endpoint was performed in a pregnant population enrolled in a prospective cohort in the DRC [74]. In this cohort 33.3% of women with *S. haematobium* had any STI. After adjusting for potential confounders, women with *S. haematobium* had increased odds of any STIs (*C. trachomatis, N. gonorrhoeae, T. vaginalis*) (aOR 3.0 (1.5 – 6.0), and specifically T. *vaginalis* (aOR 2.3 [1.1 – 4.8]) [74].

Figure 1.5 –"Urogenital schistosomiasis" and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence



Abbreviations: Ab – antibody, Assoc – association, CAA – circulating anodic antigen, diff – difference, HSV-2 – herpes simplex virus-2, IL – interleukin, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, Sh – *S. haematobium*, Sm – *S. mansoni,* STI – sexually transmitted infection

Overall, in the studies that aimed to detect *Schistosoma* infection but did not evaluate for genital involvement, sixteen studies evaluated "urogenital schistosomiasis" or "mixed/unspecified/*S. mansoni"* infection, the majority of which evaluated HIV-1 as a study endpoint (78.6%, 11/14) [18, 19, 45, 50-61, 74]. Of these, only a minority (25.0%, 3/12) show an association between schistosome infection and HIV-1, and if present, the association was observed in studies evaluating women, sub-groups of women [18, 52, 57] or in *S. haematobium* infection [18].

While the mechanism of the association between schistosomiasis and HIV-1 is still largely unknown, similar mechanistic hypotheses exist for *S. haematobium* as for *S. mansoni* and include the effects of mucosal barrier disruption as well as the impact of helminth infection on the local and systemic immune responses. *S. haematobium* infection is associated with a higher mean egg concentration in genital tissue compared with *S. mansoni* [62] a factor which may increase the probability of cervicovaginal mucosal manifestations. Additionally, *S. haematobium* infection may modulate the local and systemic immune system through changes in the cytokine environment, cervicovaginal microbiota and differential gene expression [59, 60, 75]. Indeed, distinct cervicovaginal gene expression profiles have been observed in women with *S. haematobium* infection with proposed involvement of genes relating to the inflammatory response and tissue [60].

Further support for the effect of *S. haematobium* on the local cervicovaginal immune environment comes from histopathology evaluation of biopsies from Malawian women (n=61) showed that cervical tissue containing *S. haematobium* eggs had a higher density of $CD4^+$ T-cells and macrophages than non-egg containing tissue [76]. Additionally, a small South African study (n=44) reported that compared to FGS negative women, participants with visual FGS had increased expression of the CCR5 co-receptor on plasma $CD4^+$ cells and cervical $CD14^+$ monocytes [77]. In a small study of Malawian women with *S. haematobium* on cervicovaginal biopsies compared with Norwegian (n=44) and endemic (n=25) controls, tissue containing schistosome eggs was associated with increased vascularity compared to non-endemic controls [78]. These small studies support the hypothesis that the association between *S. haematobium* and HIV-1 could be biologically plausible with *S. haematobium* egg deposition beginning an inflammatory cascade that may to mucosal disruption and a milieu supportive of HIV-1 infection [14].

1.3.4 Visual FGS

I defined studies as "visual FGS", when the presence of any of the four recognized FGS cervicovaginal manifestations was utilized as the diagnostic technique. The following cervicovaginal lesions were identified by an expert clinician during a pelvic exam: grainy sandy patches, homogenous yellow sandy patches, rubbery papules, and abnormal blood vessels [27]. Other diagnostic tests, such as urine microscopy and CVL PCR for *Schistosoma* DNA could be utilized to confirm the status of negative controls [77] or for comparison against "visual FGS" as the reference standard [26, 79].

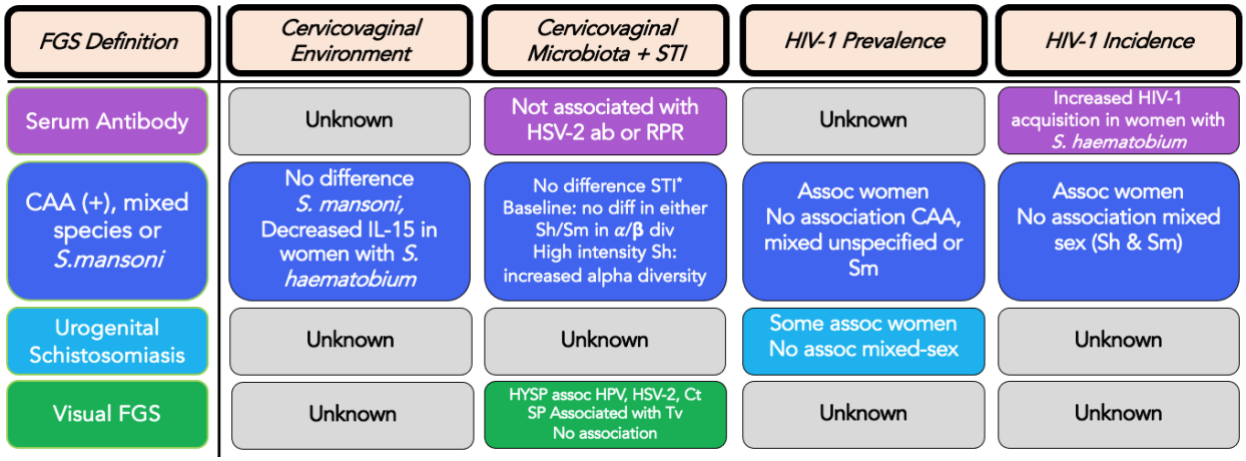
Three studies met pre-defined criteria, all of which reported STI outcomes [26, 77, 79] (Figure 1.6). A Madagascan study of 118 women with "visual FGS" did not find an association between any of the evaluated STIs (*Neisseria gonorrhoeae*, *Mycoplasma genitalium, Trichomonas vaginalis, Chlamydia trachomatis*, *Treponema pallidum,* HSV-2, or *H. ducreyi)* and the presence of rubbery papules, sandy patches, contact bleeding, or abnormal mucosal vessels [79]. A Zimbabwean study of 527 women, 46% (243/527) with "visual FGS", found that while there was weak evidence that grainy sandy patches were associated with *N. gonorrhoeae* (odds ratio [OR] 7.28 (0.75 – 70.69, p=0.09), homogeneous yellow sandy patches were associated with HPV (OR 2.35 (1.20-4.59, p=0.012), HSV-2 (OR 1.62 [1.05-2.51], p=0.03) and *Chlamydia (C.) trachomatis (OR 5.62 (1.02-*

31.12], p=0.048) [26]. A South African study of 44 women, 43.2% (19/44) of whom had "visual FGS" found strong evidence that *T. vaginalis* was associated with sandy patches (42% [8/19] visual FGS positive; 5% [1/21] visual FGS negative, p=0.007), although the sample size was small [77]. None of the identified studies reported HIV-1 prevalence or incidence outcomes.

"Visual FGS" evaluation techniques may be similar across studies as the three identified studies of "visual FGS", albeit performed in different study populations, were all conducted by the same principal investigator. However, each study reported varying evidence against the null hypothesis of the association of STIs with "visual FGS". This may be related to power, as the Madagascan [79] and South African [77] studies had smaller sample sizes than the Zimbabwean study [26]. Additionally, the STI diagnostic methods should also be considered. The South African study that detected a difference in *T. vaginalis* proportions between women with and without "visual FGS" utilized molecular methods (PCR) on CVL [77]. However, the Zimbabwean study used culture and serology and the Madagascan study used urine PCR, methods all less sensitive than molecular methods using vaginal fluid [80, 81]. It may also suggest that the reported associations between STIs and "visual FGS" are related to chance.

The authors of the identified studies propose that since there wasn't strong evidence that grainy sandy patches were associated with STIs or cervical dysplasia that grainy sandy patches can be considered "pathognomonic" for FGS [26]. However, the presence of grainy sandy patches in the Zimbabwean study was not associated with *S. haematobium* eggs on biopsy (OR 7.38 [0.79-69.29, p=0.80], though grainy sandy patches were associated with finding *S. haematobium* eggs in any genital specimen (OR 4.51 [2.71-7.52], p<0.01) [26]. Though felt to be a strong indicator of "visual FGS", clinical evidence of grainy sandy patches is not perfectly correlated with the presence of eggs on histopathology. In the Madagascan study, of 31 biopsies, 61.3% (19/31) had adequate stroma for evaluation and 73.6% (14/19) of these contained parasite eggs, with 28.6% (4/14) of these specimens being from grainy sandy patches [79]. Including the small sample size, this study illustrates some limitations to the use of "visual FGS" as a diagnostic standard in research settings.

Figure 1.6 – "Visual FGS" and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence



Abbreviations: Ab – antibody, Assoc – association, CAA – circulating anodic antigen, Ct – *Chlamydia trachomatis*, diff – difference, HPV – human papillomavirus, HSV-2 – herpes simplex virus-2, HYSP – homogeneous yellow sandy patch, IL – interleukin, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, Sh – *S. haematobium*, Sm – *S. mansoni,* SP – sandy patch, STI – sexually transmitted infection, Tp – Treponema pallidum, Tv – Trichomonas vaginalis

1.3.5 Tissue FGS (including studies using composite endpoints)

Next, I identified studies using a tissue diagnosis as the FGS diagnostic technique. These studies also reported an association between "tissue FGS" and the cervicovaginal environment, the cervicovaginal microbiota/STIs, HIV-1 prevalence or HIV-1 incidence as study endpoints. The finding of one or more terminal spined eggs in any tissue specimen was defined as a tissue diagnosis of FGS, or "tissue FGS". Various techniques were implemented across studies in making a "tissue FGS" diagnosis, including cervical wet mount, pap smear, and cervicovaginal biopsy (using either quantitative crushed biopsy technique [QCBT] or histopathology). Studies that had any proportion of women with tissue diagnoses were grouped in this category, even if the proportion was less than half of participants [17, 82] or used a composite endpoint of "urogenital schistosomiasis" and "tissue FGS". Studies that used biopsy to confirm "visual FGS" as a reference standard are already described above [26, 79].

Four studies used tissue diagnostics and reported pre-specified outcomes associated with "tissue FGS" [10, 17, 82, 83]. An additional eight studies of "tissue FGS" were identified but were not

included for the following reasons: three studies of "tissue FGS" did not investigate pre-specified outcomes [8, 11, 22] , three evaluated STIs but did not associate results with "tissue FGS" [23, 84, 85], and two evaluated STIs but associated STI results with the study site rather than "tissue FGS" [9, 86].

Of these four identified studies, two reported STI endpoints in participants with "tissue FGS" [10, 17] and three reported HIV-1 outcomes [17, 82, 83]. Overall, the included studies did not identify an association between "tissue FGS" and STIs. A small Malawian study (n=51) reported cervicovaginal biopsy results for all study participants [10] (Figure 1.7). Participant specimens with koilocytosis or positive immunofluorescence on immunohistochemistry were evaluated for HPV [10]. In 15 women, there was no difference in HPV by "tissue FGS" status (11 with and 4 without "tissue FGS", p=0.41) [10]. Similarly, in a Tanzanian study, there was no difference in the proportion of women with *C. albicans*, *T. vaginalis*, BV and *T. pallidum* with or without "tissue FGS" [17]. Of note, microscopy was used to identify *C. albicans*, *T. vaginalis*, *G. vaginalis* and this technique is less sensitive than molecular methods [80].

Studies evaluating the association between "tissue FGS" and prevalent HIV-1 have shown conflicting results. Of the four studies identified, three evaluated an association between "tissue FGS" and prevalent HIV-1 [17, 82, 83]. In a Ghanaian study, 331 women had a cervicovaginal biopsy performed and there was no association detected between "tissue FGS" status and prevalent HIV-1, 0.9% of those with "tissue FGS" had HIV-1 and 6.6% of "tissue FGS" negative women had HIV-1 [83]. However, the methods of analysis used by the authors would be more suitable for ordered categorical variable data [83]. In the two remaining studies, some proportion of the population had evidence of eggs detected in cervicovaginal tissue, 30.4% (7/23) in a Tanzanian study and 43.4% (70/445) in a Zimbabwean study [17, 82]. In the Zimbabwean study, the proportion of women with HIV-1 was higher in those with *S. haematobium* ova detected in a tissue-specimen (23.0% [29/125]) versus those without (13.0% [41/]320) (OR 2.1 [1.2-3.5], p=0.008) [82]. Likewise, in the Tanzanian study, the proportion of women with HIV-1 was higher in those with *S. haematobium* ova detected in a composite endpoint of "urogenital schistosomiasis" or tissue-specimen (17.4% [4/23]) versus those without (5.3% [23/434]) (OR 4.0 [1.2-13.5, p=0.024) [17]. The reason for discordant results in the association between HIV-1 prevalence and "tissue FGS" between the Ghanaian versus the Tanzanian and Zimbabwean studies is unclear but may be related

to a form of selection bias. Only the Ghanaian study biopsied all study participants and standardized the biopsy location to the anterior lip of the cervix, in the absence of suggestive clinical manifestations [83]. However, the Tanzanian and Zimbabwean studies performed cervical biopsy only in the setting of clinical indications [82] or abnormal cervical lesions [17] and thus they may represent an inherently different patient population. Additionally, the biopsy methods in these studies are not well-described, and thus there may be substantial heterogeneity in technique [17, 82, 83]. Background *S. haematobium* prevalence and test sensitivity can affect the number of true positive test results, and the Ghanaian study doesn't report baseline urinary *S. haematobium* prevalence, which was 10.8% (13/120) in the Tanzanian study and 39.4% (177/449) in the Zimbabwean study [17, 29, 83]. Additionally, differences in sampling methods could be considered (Ghana – quantitative compressed biopsy technique [QCBT], Tanzania – cervical smear or QCBT, Zimbabwe – cervical smear, wet mount, or biopsy (histology and QCBT)). *S. haematobium* eggs are not evenly distributed in cervicovaginal tissue and can cluster together [87] and are also found primarily in the stroma and are less likely to be found in the epithelium [23], so it is possible that *S. haematobium* cases might have been missed if biopsy technique was not optimal. No estimate of biopsy quality is provided in the Ghanaian study, however QCBT has been shown to identify more "tissue FGS" cases than standard histopathology [83, 87] . Despite their limitations, the Zimbabwean and Tanzanian studies represent what is considered in the field to be the best available evidence for the association between FGS and prevalent HIV-1 and are cited for their effect sizes and the strong evidence of an association with prevalent HIV-1, despite the small sample sizes and use of sub-groups of the larger study populations. While the effect sizes are moderate to high in the Zimbabwean and Tanzanian studies respectively, they should be considered in the context of the negative results in the Ghanaian study which had a larger sample size.

Figure 1.7 – "Tissue FGS" and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence

FGS Definition	*Cervicovaginal Environment*	*Cervicovaginal Microbiota + STI*	*HIV-1 Prevalence*	*HIV-1 Incidence*
Serum Antibody	Unknown	Not associated with HSV-2 ab or RPR	Unknown	Increased HIV-1 acquisition in women with *S. haematobium*
CAA (+), mixed species or *S.mansoni*	No difference *S. mansoni,* Decreased IL-15 in women with *S. haematobium*	No difference STI* Baseline: no diff in either Sh/Sm in α/β div High intensity Sh: increased alpha diversity	Assoc women No association CAA, mixed unspecified or Sm	Assoc women No association mixed sex (Sh & Sm)
Urogenital Schistosomiasis	Unknown	Unknown	Some assoc women No assoc mixed-sex	Unknown
Visual FGS	Unknown	HYSP assoc HPV, HSV-2, Ct SP Associated with Tv No association	Unknown	Unknown
Tissue FGS/FUS	Unknown	No difference between BV, Tv, Ca, Tp	Association described No assoc described	Unknown

Abbreviations: Ab – antibody, Assoc – association, Ca – Candida albicans, CAA – circulating anodic antigen, Ct – *Chlamydia trachomatis*, BV – bacterial vaginosis, diff – difference, HPV – human papillomavirus, HSV-2 – herpes simplex virus-2, HYSP – homogeneous yellow sandy patch, IL – interleukin, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, Sh – *S. haematobium*, Sm – *S. mansoni,* SP – sandy patch, STI – sexually transmitted infection, Tp – Treponema pallidum, Tv – Trichomonas vaginalis

1.3.6 PCR-defined FGS

Finally, studies that used PCR as the reference standard to detect *Schistosoma* DNA in genital specimens were evaluated if they assessed the following endpoints: the cervicovaginal environment, the cervicovaginal microbiota/STIs, HIV-1 prevalence, or HIV-1 incidence. Six studies implemented PCR for FGS diagnosis [25, 29, 79, 88-90]. One study used *Schistosoma* PCR results to validate findings with visual diagnosis as the reference standard and was thus reported above under "visual FGS" [79]. Four identified studies did not report the pre-specified endpoints [25, 88-90] (Figure 1.8). Thus, only one study reported independent PCR outcomes and their association with HIV-1, whilst also using "visual FGS" as a reference standard for calculating the sensitivity of PCR [29]. In this Zimbabwean study, 83 CVL specimens (18%, 83/557 of the original specimens) were processed retrospectively for *Schistosoma* DNA by PCR. In this cohort, 36.1% (30/83) of samples had *Schistosoma* DNA detected and 30 women had sandy patches, with 56% (17/30) having both detectable *Schistosoma* DNA and sandy patches [29]. While this study suggests that

PCR detection of *Schistosoma* DNA is a useful diagnostic strategy for FGS, this cohort did not identify an association between prevalent HIV-1 and *Schistosoma* DNA detection (p=0.97) [29]. Additionally, six studies implemented PCR, but did not utilize this semi-quantitative molecular method as a reference standard. This research gap will be further addressed by this PhD thesis.

Figure 1.8 – "PCR-defined FGS" and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence

FGS Definition	*Cervicovaginal Environment*	*Cervicovaginal Microbiota + STI*	*HIV-1 Prevalence*	*HIV-1 Incidence*
Serum Antibody	Unknown	Not associated with HSV-2 ab or RPR	Unknown	Increased HIV-1 acquisition in women with *S. haematobium*
CAA (+), mixed species or *S.mansoni*	No difference *S. mansoni,* Decreased IL-15 in women with *S. haematobium*	No difference STI* Baseline: no diff in either Sh/Sm in α/β div High intensity Sh: increased alpha diversity	Assoc women No association CAA, mixed unspecified or Sm	Assoc women No association mixed sex (Sh & Sm)
Urogenital Schistosomiasis	Unknown	Unknown	Some assoc women No assoc mixed-sex	Unknown
Visual FGS	Unknown	HYSP assoc HPV, HSV-2, Ct SP Associated with Tv No association	Unknown	Unknown
Tissue FGS/FUS	Unknown	No difference between BV, Tv, Ca, Tp	Association described No assoc described	Unknown
PCR-FGS	Unknown	Unknown	No association detected	Unknown

Abbreviations: Ab – antibody, Assoc – association, Ca – Candida albicans, CAA – circulating anodic antigen, Ct – *Chlamydia trachomatis*, BV – bacterial vaginosis, diff – difference, HPV – human papillomavirus, HSV-2 – herpes simplex virus-2, HYSP – homogeneous yellow sandy patch, IL – interleukin, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, Sh – *S. haematobium*, Sm – *S. mansoni,* SP – sandy patch, STI – sexually transmitted infection, Tp – Treponema pallidum, Tv – Trichomonas vaginalis

1.4 The pathogenesis of HIV-1 acquisition: the role of STIs, vaginal microbiota, and FGS

HIV-1 acquisition after sexual contact occurs primarily at mucosal surfaces where an intact cervicovaginal epithelium provides the first line of defence against pathogens [91, 92]. Both ulcerative and non-ulcerative STIs have been associated with increased odds of HIV-1 seroconversion [93, 94] suggesting that the pathophysiology of HIV-1 vulnerability extends beyond physical breaches to the cervicovaginal barrier. Indeed, STIs are associated with increased risk of

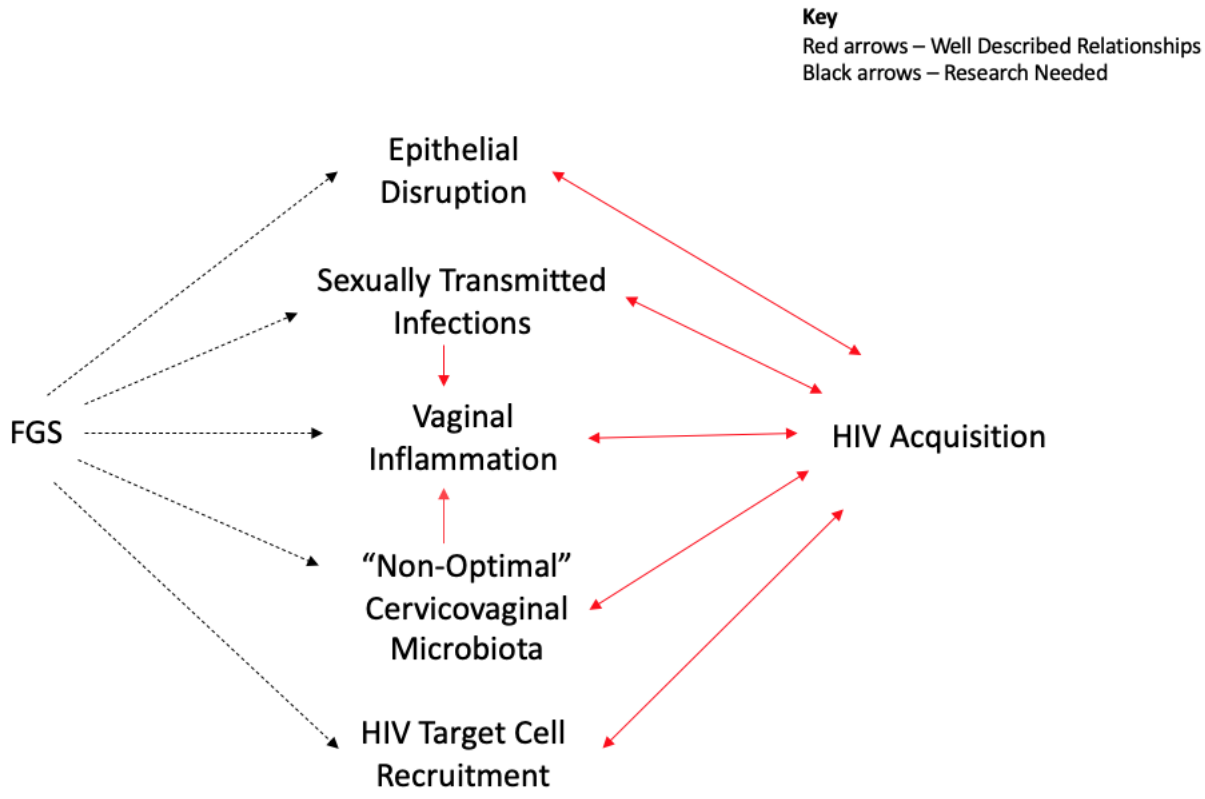
HIV-1 acquisition through a number of mechanisms, including breaches in the epithelium (e.g. lesions) and through cervicovaginal inflammation [95]. Both viral and bacterial pathogens can activate host cytokine and chemokine cascades. While these cascades are an important component of pathogen clearance, they can also result in tissue damage [96]. Thus, vaginal inflammation, or the STI-related expression of cervicovaginal cytokines and chemokines, is important in HIV-1 pathogenesis [97] because it mediates the recruitment and activation of target cells [95, 97] and is associated with increased permeability of the cervicovaginal barrier [65]. The influx of HIV-1 target cells and a damaged cervicovaginal barrier set the stage for increased HIV-1 vulnerability, but relatively little is known regarding the nature of the epithelial disruption in FGS [76, 78]. HIV-1 infection can impair cervicovaginal barrier function [65], but more research is needed to understand if epithelial disruption in FGS is associated with HIV-1 acquisition. Additionally, while the recruitment of activated HIV-1 target cells to the cervicovaginal epithelium may be associated with HIV-1 susceptibility [91, 97, 98], and acute HIV-1 infection [99], the role of FGS in the recruitment and activation of cervicovaginal HIV-1 target cells has not been well-defined [77].

Similar to STI, cervicovaginal microbiota and bacterial vaginosis (BV) may also act as a risk factor in HIV-1 acquisition [100, 101]. BV is an example of "non-optimal" microbiota, which is characterized by a shift from lactobacilli dominance to an increase in more diverse anaerobic species [102]. In contrast, mucosal surfaces have been described as "optimal" when they provide an intact barrier against pathogens [102]. In the cervicovaginal environment, protection is mediated by acid producing bacteria, most commonly lactobacilli species. These commensal microorganisms adhere to an intact vaginal squamous epithelium [102, 103], protecting against pathogens by acidifying the vagina and producing antimicrobial substances such as bacteriocins [103]. Similar to STI, more diverse bacterial communities have been associated with elevated levels of activated HIV-1 target cells and vaginal cytokines and chemokines [104]. FGS has been proposed as a risk factor for HIV-1 acquisition, though evidence both supports and refutes this hypothesis [17, 82, 83]. Well-known HIV-1 acquisition risk factors, such as STI and BV, and potential risk factors, such as FGS, are potentially modifiable.

In this PhD thesis, I present a causal framework describing how FGS may potentially be associated with mechanisms that influence HIV-1 acquisition. This causal framework suggests that FGS may be associated with conditions such as epithelial disruption, STI, vaginal inflammation, HIV-1 target

cell recruitment, or "non-optimal" cervicovaginal microbiota that have a well-described association with HIV-1. If present, these associations may mediate some of the FGS-related HIV-1 susceptibility (Figure 1.9). STI, vaginal inflammation and the cervicovaginal microbiota have not been well-defined in women with PCR-defined FGS and this PhD thesis aims to address these research gaps.

Figure 1.9 – Conceptual pathway describing the potential association of FGS with epithelial disruption, STI, vaginal inflammation and "non-optimal" cervicovaginal microbiota, and HIV-1 target cell recruitment*



**adapted from [14]*

1.5 Knowledge gaps and PhD thesis rationale

This chapter identifies a number of research gaps. Little is known about the cervicovaginal environment or cervicovaginal microbiota in women with schistosome infection, and a notable gap includes the lack of data on women with genital schistosomiasis (Figure 1.10). I did not identify any

studies reporting the prevalence and association of the cervicovaginal immune proteins, microbiota, or STIs with "PCR-defined FGS" (Figure 1.10). Additionally, while conflicting measures of incident HIV-1 have been put forward from women with evidence of *S. haematobium* by antibody testing, or antibody testing combined with CAA, to date there are no studies measuring HIV-1 incidence in "PCR-defined FGS" (Figure 1.10). While many studies have used PCR to confirm "visual FGS", this chapter illustrates that "PCR-defined FGS" has been underutilized as a semi-quantitative diagnostic method and as an independent reference standard. Thus, the overall objectives of this PhD thesis are relevant and include: to describe the cervicovaginal microbiota (including STIs) and the cervicovaginal immune environment in Zambian women with and without "PCR-defined FGS" and to explore the association of "PCR-defined FGS" with HIV-1 incidence.

Figure 1.10 – *Schistosoma* infection categories and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence with references

FGS Definition	*Cervicovaginal Environment*	*Cervicovaginal Microbiota + STI*	*HIV-1 Prevalence*	*HIV-1 Incidence*
Serum Antibody	Unknown	Not associated with HSV-2 ab or RPR[1]	Association detected[2]	Increased HIV-1 acquisition in women with *S. haematobium*[1]
CAA (+), mixed species or *S.mansoni*	No difference *S. mansoni,* Decreased IL-15 in women with *S. haematobium* [3]	No difference STI* [3,4,5] Baseline: no difference in Sh/Sm in α/β diversity [6] High intensity Sh: increased α diversity	Assoc women [7] No association CAA, mixed, unspecified, or *S. mansoni* [4, 8, 9, 10, 11, 12]	Assoc women [13] No association mixed sex (Sh & Sm) [14, 15]
Urogenital Schistosomiasis	Unknown	Assoc with STI* and *T. vaginalis* [16]	Some assoc women [17] No assoc mixed-sex [18]	Unknown
Visual FGS	Unknown	HYSP assoc HPV, HSV-2, Ct [19] SP Associated with Tv [20] No association* [21]	Unknown	Unknown
Tissue FGS/FUS	Unknown	No difference between BV, Tv, Ca, Tp [22] HPV [23]	Association detected [22, 24] No assoc detected [25]	Unknown
PCR-FGS	Unknown	Unknown	No association detected [26]	Unknown

STI*:
References 3, 5, and 16 – *C. trachomatis, N. gonorrhoeae, T. vaginalis;* (reference 5, weak evidence of an association between *S. mansoni* and *C. trachomatis* (p=0.06))
Reference 4 – "diagnosis of STI in the past 12 months"
Reference 21 – *C. trachomatis, N. gonorrhoeae, T. vaginalis, M. genitalium, T. pallidum, H. ducreyi,* and HSV-2

Abbreviations: Ab – antibody, Assoc – association, Ca – Candida albicans, CAA – circulating anodic antigen, Ct – *Chlamydia trachomatis*, BV – bacterial vaginosis, diff – difference, HPV – human papillomavirus, HSV-2 – herpes simplex virus-2, HYSP – homogeneous yellow sandy patch, IL – interleukin, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, Sh – *S. haematobium*, Sm – *S. mansoni,* SP – sandy patch, STI – sexually transmitted infection, Th2 – T-helper 2, SG – subgroup, Tp – Treponema pallidum, Tv – Trichomonas vaginalis

References:

1. Wall KM, Kilembe W, Vwalika B, et al. Schistosomiasis is associated with incident HIV transmission and death in Zambia. PLoS Negl Trop Dis **2018**; 12:e0006902.
2. Stabinski L, Reynolds SJ, Ocama P, et al. High prevalence of liver fibrosis associated with HIV infection: a study in rural Rakai, Uganda. Antivir Ther **2011**; 16:405-11.
3. Dupnik KM, Lee MH, Mishra P, et al. Altered Cervical Mucosal Gene Expression and Lower Interleukin 15 Levels in Women With Schistosoma haematobium Infection but Not in Women With Schistosoma mansoni Infection. The Journal of infectious diseases **2019**; 219:1777-85.
4. Kapiga S, Hansen CH, Downs JA, et al. The burden of HIV, syphilis and schistosome infection and associated factors among adults in the fishing communities in northwestern Tanzania. Trop Med Int Health **2021**; 26:204-13.
5. Yegorov S, Galiwango RM, Good SV, et al. Schistosoma mansoni infection and socio-behavioural predictors of HIV risk: a cross-sectional study in women from Uganda. BMC Infect Dis **2018**; 18:586.
6. Bullington BW, Lee MH, Mlingi J, et al. Cervicovaginal bacterial communities in reproductive-aged Tanzanian women with Schistosoma mansoni, Schistosoma haematobium, or without schistosome infection. ISME J **2021**.
7. Downs JA, van Dam GJ, Changalucha JM, et al. Association of Schistosomiasis and HIV infection in Tanzania. Am J Trop Med Hyg **2012**; 87:868-73.
8. Mazigo HD, Dunne DW, Wilson S, et al. Co-infection with Schistosoma mansoni and Human Immunodeficiency Virus-1 (HIV-1) among residents of fishing villages of north-western Tanzania. Parasites & vectors **2014**; 7:587.
9. Sanya RE, Muhangi L, Nampijja M, et al. Schistosoma mansoni and HIV infection in a Ugandan population with high HIV and helminth prevalence. Trop Med Int Health **2015**; 20:1201-8.

10. Fontanet AL, Woldemichael T, Sahlu T, et al. Epidemiology of HIV and Schistosoma mansoni infections among sugar-estate residents in Ethiopia. Annals of tropical medicine and parasitology **2000**; 94:145-55.
11. Kallestrup P, Zinyama R, Gomo E, et al. Schistosomiasis and HIV-1 infection in rural Zimbabwe: implications of coinfection for excretion of eggs. The Journal of infectious diseases **2005**; 191:1311-20.
12. Downs JA, de Dood CJ, Dee HE, et al. Schistosomiasis and Human Immunodeficiency Virus in Men in Tanzania. Am J Trop Med Hyg **2017**; 96:856-62.
13. Downs JA, Dupnik KM, van Dam GJ, et al. Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: A nested case-control study. PLoS Negl Trop Dis **2017**; 11:e0005968.
14. Bochner AF, Baeten JM, Secor WE, et al. Associations between schistosomiasis and HIV-1 acquisition risk in four prospective cohorts: a nested case-control analysis. J Int AIDS Soc **2020**; 23:e25534.
15. Ssetaala A, Nakiyingi-Miiro J, Asiki G, et al. Schistosoma mansoni and HIV acquisition in fishing communities of Lake Victoria, Uganda: a nested case-control study. Trop Med Int Health **2015**; 20:1190-5.
16. Gadoth A, Mvumbi G, Hoff NA, et al. Urogenital Schistosomiasis and Sexually Transmitted Coinfections among Pregnant Women in a Schistosome-Endemic Region of the Democratic Republic of Congo. Am J Trop Med Hyg **2019**; 101:828-36.
17. Ndhlovu PD, Mduluza T, Kjetland EF, et al. Prevalence of urinary schistosomiasis and HIV in females living in a rural community of Zimbabwe: does age matter? Trans R Soc Trop Med Hyg **2007**; 101:433-8.
18. N'Zoukoudi-N'Doundou MY, Dirat I, Akouala JJ, Penchenier L, Makuwa M, Rey JL. [Bilharziasis and human immunodeficiency virus infection in Congo]. Med Trop (Mars) **1995**; 55:249-51.
19. Kjetland EF, Ndhlovu PD, Mduluza T, et al. Simple clinical manifestations of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2005**; 72:311-9.
20. Kleppa E, Ramsuran V, Zulu S, et al. Effect of female genital schistosomiasis and anti-schistosomal treatment on monocytes, CD4+ T-cells and CCR5 expression in the female genital tract. PloS one **2014**; 9:e98593.
21. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, et al. Gynecological manifestations, histopathological findings, and schistosoma-specific polymerase chain reaction results among women with Schistosoma haematobium infection: a cross-sectional study in Madagascar. The Journal of infectious diseases **2015**; 212:275-84.
22. Downs JA, Mguta C, Kaatano GM, et al. Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region. Am J Trop Med Hyg **2011**; 84:364-9.
23. Kjetland EF, Poggensee G, Helling-Giese G, et al. Female genital schistosomiasis due to Schistosoma haematobium. Clinical and parasitological findings in women in rural Malawi. Acta Trop **1996**; 62:239-55.
24. Kjetland EF, Ndhlovu PD, Gomo E, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS **2006**; 20:593-600.

25. Yirenya-Tawaiah D Amoah C Apea-Kubi K Dade M Annang T Bosompem KM. HIV testing in community-based research: a case study of female genital schistosomiasis and HIV in the Volta Basin of Ghana. J Ghana Sci Assoc. 2009; 11: unpaginated. Available at: https://www.ajol.info//index.php/jgsa/article/view/46997.
26. Kjetland EF, Hove RJ, Gomo E, et al. Schistosomiasis PCR in vaginal lavage as an indicator of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2009**; 81:1050-5.

Table 1.1a –Literature describing HIV-1 prevalence or HIV-1 incidence in participants with *Schistosoma* antibody detected

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	Proportion with HIV-1 Infection	Proportion with Schistosome Infection	HIV Infection in participants with Schistosome Infection
Stabinski 2011 [48]	Uganda	Cross Sectional	1000	Women 67.0% (670/ 1000)	Mean (range) HIV (+) 38 (31-44) HIV (-) 37 (32-44)	Serum soluble egg antigen ELISA	HIV (+) 50% (500/1000)	*Schistosoma* antibody (+) % 11.7 (117/1000)	Schistosome infection prevalence higher in HIV (+) 14·0% (71/500) than HIV (-) 9·0% (46/500), (p=0·014)
Wall [47]	Zambia	Case-control nested within a cohort study	2,145	Women 51.2% (1099/ 2145)	Mean (SD) HIV(+) SAb(+) 28.2 (7.3) HIV(+) SAb(-) 28.3 (6.9) HIV(-) SAb(+) 27.6 (7.0) HIV(-) Sab (-) 26.6 (6.9)	Serum antibodies to schistosome soluble worm antigen preparation ELISA Species-specific antigens to differentiate *S. mansoni* and *S. haematobium* by immunoblot	55.6% (1195/2150)	*Schistosoma* antibody (+) % 58.8 (1261/2145)	There were no differences in the distribution of the ELISA results when stratifying by sex and HIV-1 status simultaneously. *S. haematobium* specific antibodies (aHR 1.4, p=0.034) increased the risk of HIV acquisition in women, while S. *mansoni* specific antibodies also increased the risk of HIV-1 acquisition, though not significantly (aHR 1.3, p=0.12)

Table 1.1b –Literature describing HIV-1 prevalence or HIV-1 incidence in unspecified schistosome infection

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis **	Proportion with HIV-1 Infection	Proportion with Schistosome Infection	HIV Infection in participants with Schistosome Infection
Downs 2012 [52]	Tanzania	Cross Sectional	345	Women 100·0% (345/ 345)	Median (IQR) 30 (24-35)	Phlebotomy (serum CAA detection)	HIV (+) 6·1% (21/345)	CAA (+) 53·6% (185/345) CAA (–) 46·4% (160/345)	Among CAA (+) women, HIV-1 infection was 9·2% (17/185) compared with 2·5% (4/160) in CAA (–) (adjusted OR = 6·2 [95% CI = 1·7-22·9], p=0·006)
Ssetaala 2015 [55]	Uganda	Case-control nested within a cohort study	200	Women 44·0% (88/ 200)	Median (IQR) 25 (22-30)	3 stool samples – KK or FME Stored serum for CAA @ 6, 12, 18 months. (+) serum CAA ≥ 32 pg/mL	HIV-1 (+) 25·0% (50/200) ††	CAA (+) at cohort enrolment 51% (102/200) CAA (+) at other timepoints: 6 months - 48·0% (96/200) 12 months - 52·0% (104/200) 18 months - 52·0% (104/200)	HIV-1 infection 49·0% (24/50) of cases and 52·0% (78/150) of controls had *S. mansoni* (CAA (+) infection prior to HIV-1 seroconversion (or a similar study visit for controls) (aOR 1·23 [95% CI 0·3-5·7], p=0·79))
Downs 2017 [57]	Tanzania	Case-control nested within a cohort survey	338	Cases: Women 61·6% (45/73) Controls: Women 61·1% (162/ 265)	Cases: median (IQR) 35 (25-43) Controls: median (IQR) 34 (25-44)	Capillary blood (CAA from dried blood spots before & after HIV-1 seroconversion) #	HIV-1 (+) 21·6% (73/338) ††	Total CAA (+) 34·0% (115/338) Women CAA (+) 32·8% (68/207) Men CAA (+) 35·8% (47/131)	Women: 44·0% (20/45) HIV-1 seroconverters were CAA (+) compared to 30·0% (48/162) HIV-1 (–) controls (OR=2·8 [95% CI, 1·2-6·6], p=0·01) Men: 29·0% (8/28) HIV-1 seroconverters were CAA (+) compared to 38·0% (39/103) HIV-1 (–) controls (OR 0·7 [0·3-1·8], p=0·42)
Kapiga 2020 [58]	Tanzania	Cross sectional	1121	Women 47.9% (537/1121)	>=18	Stored serum for CAA	HIV (+) 14.1% (159/1121)	CAA (+) all participants 83.1% (80.8-85.3) 924/1121 Recreational facility workers (100% female) 77.3% (71.9-82.1) 214/286	Schistosome infection (all): 15.0% (66/434) HIV (+) and "highly positive" (CAA) compared to 12.0% (22/190) (OR=1.37 [95% CI, 0.82-2.29], p=0.49) There was not strong evidence of a difference by participant sex

								Fisherman or boat crew (99% male) 92.7% (86.2-92.8) 319/344 General population (54% male) 81.6% (76.3-86.2) 209/253	

Table 1.1c –Literature describing HIV-1 prevalence or HIV-1 incidence in mixed schistosome infection

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	Proportion with HIV-1 Infection	Proportion with Schistosome Infection	HIV Infection in participants with Schistosome Infection
Kallestrup 2005 [51]	Zimbabwe	Cross Sectional	1545	Women 75·2% (1162/ 1545)	Median (IQR) 36·2 (25·8 - 46·4)	Fixed volume urine (3 consecutive days) One stool sample/MFE (1g stool)	HIV (+) 26·3% (407/1545)	Any Schistosome infection (by urine or stool) 43·4% (670/1545) No Schistosome infection 56·6% (875/1545) Urine (+) (*S. haematobium)* infection only 27·6% (426/1545) Stool (+) (*S. mansoni)* infection only 7·8% (121/1545) Urine and stool (+) *(S. haematobium* and *S. mansoni*) infection 8·0% (123/1545)	Among 407 HIV-1 (+) participants, 222 (54·5%) were Schistosoma uninfected, 110 (27·0%) had *S. haematobium* infection only, 40 (9·8%) had *S. mansoni* infection only, and 35 (8·6%) were infected with both *S. haematobium* and *S. mansoni* Corresponding figures among 1138 HIV-1 (–) participants were 653 (57·4%), 316 (27·8%), 81 (7·1%) and 88 (7·7%), respectively Overall p-value for association between HIV-1 and Schistosome infection was 0·31
Downs 2017 [56]	Tanzania	Cross Sectional	674	Men 100·0% (674/ 674)	Median (IQR) 34 (25-42)	10mL urine 1 stool sample – 5 KK slides (41.7mg/slide) Phlebotomy for CAA (positive ≥30 pg/mL) <u>Definitions:</u> S. *mansoni* infection: eggs in stool or CAA (+) with no *S.*	HIV-1 (+) 5·6% (38/674)	CAA (+) or Egg (+) 63·6% (429/674) *S. mansoni* (Stool (+) or serum CAA (+) with Urine (–)) 49·9% (336/674) *S. haematobium* (Urine (+) or serum CAA (+) with Stool (–)) 14·1% (95/674) *S. mansoni* Stool (+) 34·4% (232/674)	In HIV-1 (+), 68·4% (26/38) had any schistosome infection (CAA (+) or egg (+)) compared with 63·4% (403/636) among HIV-1 (–) (OR=1·3 [0·6-2·5, p=0·53]) In HIV (+), 5·3% (2/38) were *S. haematobium* egg (+) compared with 5·5% (35/636) among HIV-1 (–) (OR not calculated due to small numbers, p=1·0) In HIV (+), 29·0% (11/38) were *S. mansoni* egg (+) compared with 34·8% (221/636) among HIV-1 (–) (OR=0·8; [95% CI 0·4- 1·9], p=0·63)

						haematobium eggs in urine *S. haematobium* infection: eggs in urine or CAA (+) with no *S. mansoni* eggs in stool		*S. haematobium* Urine (+) 5·5% (37/674)	
Bochner 2020 [45]	Kenya and Uganda	Case-control nested within a longitudinal study	245 cases & 713 controls (serodiscordant) 330 cases & 962 controls (sex worker)	Serodiscordant study 41.1% Women (394/958) Female sex worker cohort 100% women n=1292	>=16	Three-stage testing algorithm: All samples tested by SEA ELISA SEA-positive samples tested by serum CAA Samples SEA and CAA positive were tested by species specific immunoblots	Serodiscordant study 245 cases & 713 controls Female Sex worker cohort 330 cases and 962 controls	Serodiscordant couple cohort, 32% (305/958) were antischistosomal (SEA) antibody positive 64% (194/305) tested positive for schistosome antigens (CAA) Female sex worker (FSW) cohort, 34% (439/1292) were SEA antibody positive 66% (290/439) tested antigen positive	No association between schistosomiasis and HIV-1 acquisition risk in males (aOR 0.99, 95% CI 0.59-1.67) or females (aOR 1.21, 95% CI 0.64-2.30) in serodiscordant couples. No association between schistosomiasis and HIV-1 acquisition risk in female sex workers aIRR 1.11, 95% CI 0.83-1.50) There was no 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).

Table 1.1d –Literature describing HIV-1 prevalence or HIV-1 incidence in *S. mansoni* infection

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	Proportion with HIV-1 Infection	Proportion with Schistosome Infection	HIV Infection in participants with Schistosome Infection
Fontanet 2000 [50]	Ethiopia	Cross Sectional	1239	Women 28·2% (349/ 1239)	Mean (range) 30 (15-60)	One stool sample/ 1 KK smear (41mg stool) Urine CCA performed in a subset (n=287). (+) urine CCA >30 ng AWA-TCA/mL	HIV (+) 4·2% (52/1239)	Stool (+) *(S. mansoni)* 31·4% adjusted prevalence (358/1239) † Urine CCA (+) 49·8% (143/287)	Schistosome infection equally prevalent among HIV (–) 31·6% (348/1187) and HIV (+) 25·1% (10/52), (adjusted OR 1·01 (p=0·98))
Mazigo 2014 [53]	Tanzania	Cross Sectional	1785	Women 52·9% (945/ 1785)	Mean (SD) 35·6 (9·74)	One stool sample/KK – 4 smears (47·1 mg)	HIV-1 (+) 7·0% (125/1785)	Stool (+) *(S. mansoni)* 47·9% (854/1785)	In HIV-1 (–) the prevalence of *S. mansoni* (+) was 48·1% (804/1660) compared with HIV-1 (+) individuals 39·5% (50/125) (aPR 1·01, [95% CI 0·84-1·21], p=0·93)
Sanya 2015 [54]	Uganda	Cross Sectional	1412	Women 42·0% (593/ 1412)	Mean (SD) 30·3 (9·5)	Single stool sample – 2 KK smears Urine CCA performed in a subset (n=650)	HIV-1 (+) 17·3% (244/1412)	Stool (+) (*S. mansoni)* 57·2% (719/1257) CAA (+) 73·5% (478/650)	In Stool (+) 16·1% (116/719) were HIV-1 (+) compared with 17·8% (96/538) in Stool (–) (aOR=1·04; [95% CI 0·74 - 1·47], p=0·81) In CAA (+) 20·7% (99/478) were HIV-1 (+) compared with 18·0% (31/172) who were CAA (–) (aOR=1·53; [95% CI 0·78-3·00], p=0·19)

Table 1.1e – Studies investigating the association of *unspecified/mixed/S. mansoni* infection with cervicovaginal microbiota outcomes

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	Cervicovaginal microbiota outcome
Bullington 2021 [59]	Tanzania	Longitudinal	44 (*S. haematobium* [n= 16] and controls [n=27])	Women 100.0%	*S. haematobium* infected 34 (22-41) *S. haematobium* uninfected 27.5 (23-40)	Serum CAA Urine Microscopy 1 stool sample - Kato Katz x5	At baseline, alpha diversity (1.83 vs 1.36, p=0.39) and beta diversity (p=0.60) were not different between *S. haematobium* infected and uninfected women. However, in women with high-intensity *S. haematobium* infections, there was strong evidence of higher alpha diversity than in *S. haematobium* negative participants (2.43 vs 1.37, p=0.016).
Bullington 2021 [59]	Tanzania	Longitudinal	91 (*S. mansoni* [n=39] and controls [n=52])	Women 100.0%	*S. mansoni* infected 29 (23-37) *S. mansoni* uninfected 32 (24-41)	Serum CAA Urine Microscopy 1 stool sample - Kato Katz x5	Comparing *S. mansoni* and *S. mansoni* negative women at baseline, alpha diversity (2.03 vs 1.88, p=0.32) and beta diversity (p=0.515) were not different between groups.

Table 1.1f – Studies investigating the association of *unspecified/mixed/S. mansoni* infection with cervicovaginal cytokines and chemokines

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	Immunologic Outcome
Dupnik 2019 [60]	Tanzania	Longitudinal	57 (*S. haematobium* [n= 18] and controls [n=11])	Women 100.0%	*S. haematobium* infected 21.5 (20-31) *S. haematobium* uninfected 30 (26-35)	Serum CAA Urine Microscopy 1 stool sample - Kato Katz x5	Decreased IL-15 in women with urogenital *S. haematobium* compared with women without infection.
Dupnik 2019 [60]	Tanzania	Longitudinal	40 (*S. mansoni* [n=11] and controls [n=29])	Women 100.0%	*S. mansoni* infected 29 (27-35) *S. mansoni* uninfected 32 (29-38)	Serum CAA Urine Microscopy 1 stool sample - Kato Katz x5	No difference in cervicovaginal cytokines in women with and without *S. mansoni* infection.

Table 1.1g –Literature describing the association of *unspecified/mixed/S. mansoni* infection with sexually transmitted infection

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	STI Outcomes
Yegorov 2018 [61]	Uganda	Cross Sectional	58	Women 100.0%	Mean (range) 27.5 (23.8 – 32.0)	CCA urine Stored plasma for SEA	Detection of any classical STI (defined as Ng, Ct or Tv) was not associated with schistosomiasis, all 5 Ct-infected participants were co-infected with schistosomiasis (Fisher's exact p = 0.064).
Dupnik 2019 [60]	Tanzania	Longitudinal	57 (*S. haematobium* [n= 18] and controls [n=11])	Women 100.0%	*S. haematobium* infected 21.5 (20-31) *S. haematobium* uninfected 30 (26-35)	Serum CAA Urine Microscopy 1 stool sample - Kato Katz x5	Among a subset of cervical swab specimens available for testing, there were no significant differences in the prevalence of chlamydia, gonorrhea, or trichomoniasis between those with and those without schistosome infection.
Kapiga 2020 [58]	Tanzania	Cross sectional	1121	Women 47.9% (537/1121)	>=18	Stored serum for CAA	Schistosome infection was not significantly associated with a history of genital discharge, genital sore or a diagnosis of STI in the past 12 months.

Table 1.1h – Literature describing HIV-1 prevalence or HIV-1 incidence in urogenital schistosomiasis

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	Proportion with HIV-1 Infection	Proportion with Schistosome Infection	HIV Infection in participants with Schistosome Infection
Zoukoudi 1995 [19]	Congo	Cross Sectional	895	Women 56·4% (505/ 895)	≥16	10 mL urine. If urine (–), then AWA-TCA serology (titre (+) at 1/160)	HIV (+) 5·5% (49/895)	Urine (+) *S. haematobium* 17·4% (156/895) Urine (–) Serology (+) 20·6% (185/895) Urine (+) or Serology (+) 38·1% (341/895) Urine (–) Serology (–) 61·9% (554/895)	In those with Urine (+) HIV-1 infection was 3.2% (5/156) compared with Urine (–) 5·9% (44/739), p=0·18 *** In those with Serology (+) HIV-1 infection was 3·8% (7/185) compared with Serology (–) 6·7%, (37/554), p=0·1*** In those with Urine (+) and Serology (+) HIV-1 infection was

									3·5%, (12/34) compared with Urine (–) and Serology (–), 6·7% (37/554), p=0·008
Ndhlovu 2007 [18]	Zimbabwe	Cross Sectional	544	Women 100.0%	15-49	Urine samples collected 3 consecutive days and examined by filtration technique	HIV (+) 28.7% (156/544)	Urine (+) *S. haematobium* 39.7% 216/544 Urine (–) *S. haematobium* 60.3% 328/544	In those with Urine (+) HIV-1 infection was 33.3% (72/216) compared with Urine (–) 25.6% (84/328), p=0.053 In those older than 35 years, those with Urine (+) HIV-1 infection was % 37.5 (33/88) compared with Urine (–) %16.8 (30/179), p<0.001

Table 1.1i –Literature describing the association of urogenital schistosomiasis with sexually transmitted infection

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	STI Outcomes
Gadoth 2019 [74]	Congo	Cross sectional study within a prospective cohort	367	Women 100.0%	18 years or older	Three urine samples over three days	32.0% of women with an STI had S. h infection. 33.3% of women with *S. haematobium* had an STI. After adjusting for potential confounders, women with *S. haematobium* had increased odds of any STI (Ct, Ng, Tv) (aOR 3.0 (1.5 – 6.0) Women with *S. haematobium* had increased odds of T. *vaginalis*

Table adapted from [25]
**samples collected are per participant
***No difference when analyzed by sex (p – value not given)
† Stool (+) prevalence was adjusted for the survey design, but for Urine (+), unadjusted prevalence is presented since no adjusted estimates were reported
†† Participants were selected as cases and controls on the basis of their HIV-1 status
# Threshold for positivity not reported

Abbreviations: aOR – (adjusted odds ratio), AWA-TCA – trichloroacetic-acid-soluble fraction of the adult worm antigen (contains approximately 3% CCA, Ct – *Chlamydia trachomatis*, CCA – circulating cathodic antigen, FGS – female genital schistosomiasis, FUS – female urogenital schistosomiasis, HIV – human immunodeficiency virus, KK – Kato Katz, MFE – modified formol-ether concentration, Ng – *Neisseria gonorrhoeae*, OR – odds ratio, TV – *Trichomonas vaginalis*

Chapter 2

Research Paper 1

Chapter 2 – Beyond the barrier: female genital schistosomiasis as a potential risk factor for HIV-1 acquisition.

Citation: Sturt AS, Webb EL, Francis SC, Hayes RJ, Bustinduy AL. Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition. Acta Trop **2020**; 209:105524.

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Surname/Family Name	Sturt		
Thesis Title	Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition		
Primary Supervisor	Amaya Bustinduy		

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Date	April 30, 2021

Acta Tropica 209 (2020) 105524

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition

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ARTICLE INFO

Keywords:
Schistosoma haematobium
Urogenital schistosomiasis,
Vaginal or cervicovaginal microbiota
Sexually transmitted infection
Vaginal or cervicovaginal inflammation

ABSTRACT

Female genital schistosomiasis (FGS) results from egg-deposition in the female reproductive tract primarily by the waterborne parasite *Schistosoma (S.) haematobium*, and less commonly by *Schistosoma (S.) mansoni*. FGS affects an estimated 20-56 million women worldwide, mostly in sub-Saharan Africa. There is cross-sectional evidence of increased HIV-1 prevalence in schistosomiasis-infected women, but a causal relationship between FGS and either HIV-1 acquisition or transmission has not been fully established. Beyond the pathognomonic breach in the cervicovaginal barrier caused by FGS, this narrative review explores potential mechanisms for a synergistic relationship between *S. haematobium* infection, FGS, and HIV-1 acquisition through vaginal inflammation and target cell recruitment.

1. Introduction

There are an estimated 82 million African women living with *Schistosoma* infections (Lai et al., 2015). Though the risk factors for human immunodeficiency virus-1 (HIV-1) and *Schistosoma haematobium* are different, these two conditions share a substantial geographic overlap (Figure 1A and 1B). Worldwide, 53% of people living with HIV-1 reside in Eastern and Southern Africa, where the HIV-1 epidemic disproportionately affects young women (UNAIDS 2018). In 2017, 52% of the 36.9 million people living with HIV-1 were women aged 15 years and over (UNAIDS 2018). The synergy of many co-factors present in Africa increases the risk of heterosexual HIV-1 acquisition, namely high prevalence of bacterial vaginosis and sexually transmitted infection (STI) (Cohen et al., 2012), low uptake of circumcision in adult men (Kaul et al., 2015), baseline immune activation, and endemic co-infections (Modjarrad, 2010). In addition, schistosome infection in women (Downs et al., 2012), and more specifically, female genital schistosomiasis (FGS), has been associated with prevalent HIV-1 infection (Kjetland et al., 2006; Downs et al., 2011).

This review will focus on FGS and its causative agent, the parasite *S. haematobium*, which requires an aquatic environment and a freshwater snail vector to complete its life-cycle (Colley et al., 2014). When fertilized *S. haematobium* eggs are shed in human urine and enter fresh water, the miracidia hatch and penetrate a snail host. After 4-6 weeks, cercariae penetrate human skin and enter the bloodstream (Colley et al., 2014). *S. haematobium* eggs are often laid in the vesicular plexus where proximity to the pelvis' venous drainage provides circulating eggs access to male and female urogenital tissues. FGS occurs when circulating *S. haematobium* eggs lodge in the reproductive organs. FGS has been associated with poor reproductive health outcomes including ectopic pregnancy (Helling-Giese et al., 1996), abortion (Helling-Giese et al., 1996), and infertility (Kjetland et al., 2010), likely as a consequence of parasite egg deposition and inflammation in reproductive tissues. It is hypothesized that the relationship between FGS and HIV-1 acquisition is causal, with the mucosal breach caused by FGS allowing HIV-1 access to susceptible submucosal target cells. However, there have been no longitudinal studies investigating the mechanistic links between FGS and HIV-1 acquisition. This review explores potential mechanisms for the relationship between *S. haematobium*, FGS, and HIV-1 acquisition beyond a breach in cervicovaginal mucosal barrier function.

2. Methods

2.1. Search Criteria

References for this narrative review were identified through searching PubMed and Medline databases up to February 4, 2020 with

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https://doi.org/10.1016/j.actatropica.2020.105524
Received 15 November 2019; Received in revised form 30 April 2020; Accepted 30 April 2020
Available online 13 May 2020

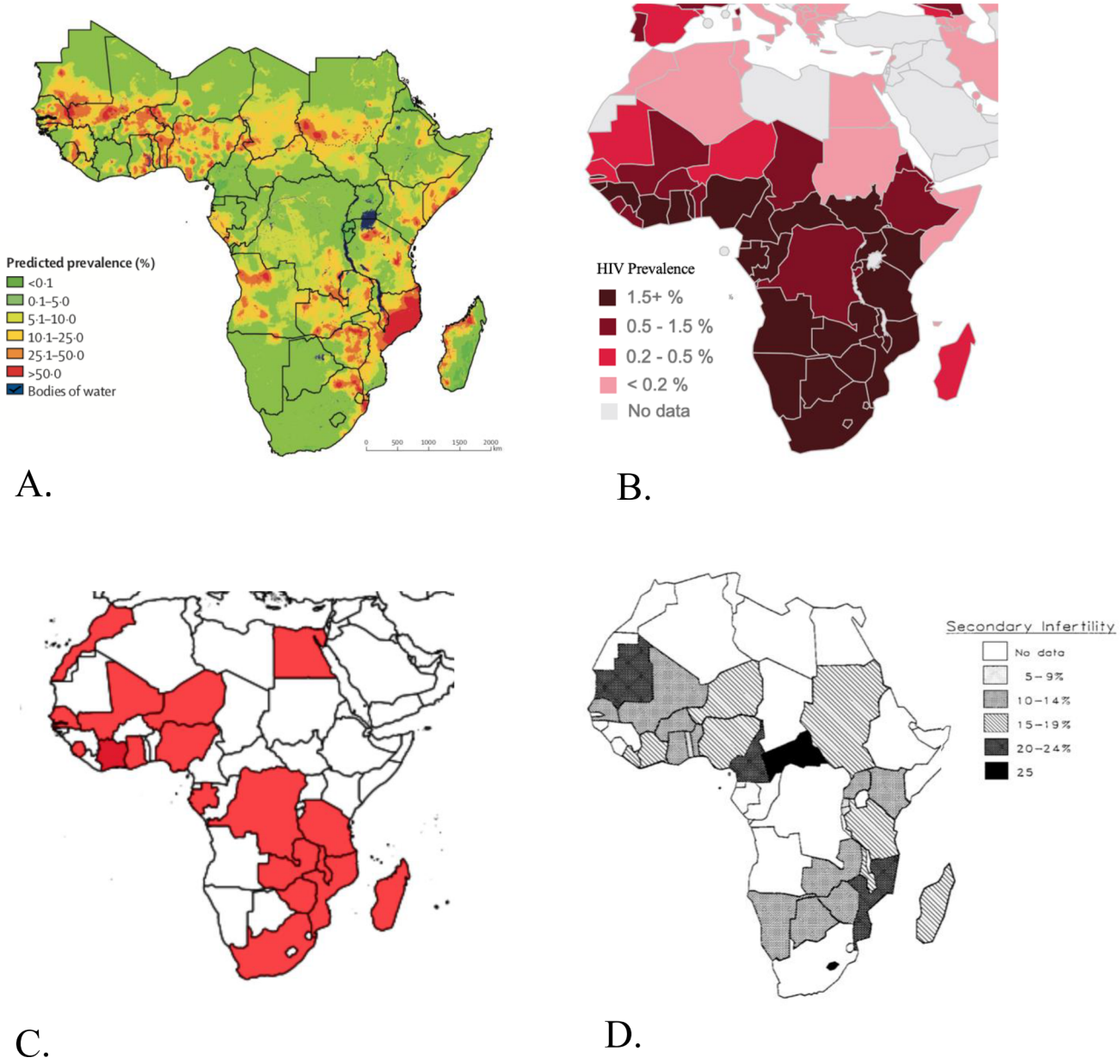

Figure 1. Overlapping geographical distributions of schistosomiasis infection, HIV-1 prevalence, reported cases of FGS, and infertility. (A) Prevalence of *S. haematobium* infection in school-aged children in Sub-Saharan Africa from 2000 onwards (from (Lai et al., 2015)); (B) HIV prevalence in African adults (15-49) (from (UNAIDS 2018)); (C) African countries with published reports of FGS (adapted from (Christinet et al., 2016)), FGS cases have been published by countries shaded in red; (D) Secondary infertility in Sub-Saharan Africa (from (Larsen 2000)).

the use of terms "Schistosomiasis" OR "*Schistosoma*" OR "Female Urogenital Schistosomiasis" OR "Genital or Urogenital Schistosomiasis" OR "Genital or Cervicovaginal Inflammation", AND "HIV or Human Immunodeficiency Virus". The references of relevant articles were also used to identify additional sources. No language restrictions were applied.

2.2. Selection Criteria

To be eligible for this narrative review (Gasparyan et al., 2011), studies must have included some proportion of women who had a definitive diagnosis of FGS, including either detection of *Schistosoma (S.)* DNA by molecular methods or through histopathologic diagnosis (eggs of either *S. haematobium* or *S. mansoni* detected in genital tissue). Studies were also eligible for inclusion if they contained a comparator group for both prevalent HIV-1 and FGS and were prospective (randomized or non-randomized) or cross-sectional studies in African populations. Initially, we searched for studies that evaluated the association between prevalent HIV-1 and FGS, but only identified two studies that fit these criteria (Supplementary Table 1). We therefore added an additional question regarding the association of *non-genital* schistosomiasis with prevalent HIV-1 in African populations, with analogous inclusion criteria to the first study question; however, with the requirement that some proportion of the study population have a measure of active schistosome infection (defined by positive microscopy or parasite circulating anodic antigen (CAA) or circulating cathodic antigen. We identified ten studies with relevant information on this question (Supplementary Table 2).

Beyond the study questions discussed above, where studies focusing on FGS were not identified, we include findings from publications reporting *S. haematobium* infection or urogenital schistosomiasis as a narrative synthesis. In circumstances where *S. haematobium* data were

not available, we reference literature relating to *S. mansoni*.

2.3. Data Extraction & Synthesis

One reviewer identified the eligible articles (A.S.) and two reviewers (A.S. and E.W.) extracted relevant data. We extracted study location, study design, participant ages, the author's definition of FGS, proportions of study participants with FGS/schistosome infection, proportion of study participants with HIV-1, method of *Schistosoma* egg detection, and the association between FGS/schistosome infection and HIV-1. Studies relating to our primary question of the association of FGS with HIV-1 are shown in Supplementary Table 1. Studies relating to the association of non-genital schistosome infection with HIV-1 are shown in Supplementary Table 2. Other articles identified in the search are referenced herein.

3. Female Genital Schistosomiasis – Definition, Clinical Presentation & Impact

3.1. Urinary schistosomiasis, genital schistosomiasis and their overlap

A working group from the World Health Organization coined the term "urogenital schistosomiasis" in 2009 to describe the frequent co-existence of *S. haematobium* ova in the urinary tract (urinary schistosomiasis) and the female genital tract (genital schistosomiasis) (World Health Organization 2009). Female Genital Schistosomiasis (FGS) refers therefore, to the presence of *S. haematobium* eggs, DNA, or characteristic clinical changes specifically in the genital tract (Figure 2), regardless of whether or not these are present in the urinary tract (Kjetland et al., 1996; Kjetland et al., 2005). Female Urogenital Schistosomiasis (FUS) refers more broadly to the deposition of schistosome eggs in either the female urinary or genital tracts, or both. FGS is caused most frequently by the parasite *S. haematobium*. *S. mansoni* eggs have, albeit rarely, also been found in genital tissue (Downs et al., 2011).

3.2. The burden of Female Genital Schistosomiasis

With up to 163 million Africans infected with *S. haematobium* or *S. mansoni* (Lai et al., 2015), schistosomiasis morbidity is an underestimated public health problem in many parts of sub-Saharan Africa (World Health Organization 2017). In the 2017 Global Burden of Disease Study, schistosomiasis was estimated to cause the loss of 1,440 million disability-adjusted life years (DALYs) (Global Burden of Disease DALYs and Hale Collaborators 2018). Many women acquired *S. haematobium* infection in childhood, and 30 to 75% of infected women may develop FGS (Leutscher et al., 1998; Kjetland et al., 2005). A study in Tanzania reported that 43% (53/122) of women with urinary schistosomiasis had concurrent FGS (diagnosed by cervical biopsy) (Poggensee et al., 1998). A cross-sectional study of girls aged 10-12 in KwaZulu-Natal, South Africa reported that significantly more girls with urinary *S. haematobium* infection reported gynecologic symptoms (bloody or foul-smelling vaginal discharge) and genital discomfort (prior to sexual debut and menstruation in 98.6% and 93.0%, respectively) (Hegertun et al., 2013). To our knowledge no clinical studies have evaluated cervicovaginal physical exam findings in school-aged girls. However, this study suggested that girls with urinary *S. haematobium* infection may develop the genital changes associated with FGS prior to sexual debut or the onset of menstruation. Since FGS may develop in childhood, it is surprising that with the prevalence of *S. haematobium* estimated at up to 25% in a survey of school-age children in a majority of sub-Saharan African countries (Lai et al., 2015), less than half of the countries (shaded red in Figure 1C) have formally published FGS case estimates. No population prevalence studies have been performed but best estimates are that 20 - 56 million women in sub-Saharan Africa may be living with FGS (World Health Organization 2017).

3.3. FGS morbidity and clinical presentation

Studies of FGS histopathology reported that women ultimately diagnosed with FGS initially sought care for vaginal bleeding, abdominal pain, or infertility (Swai et al., 2006). Frequently associated, but not

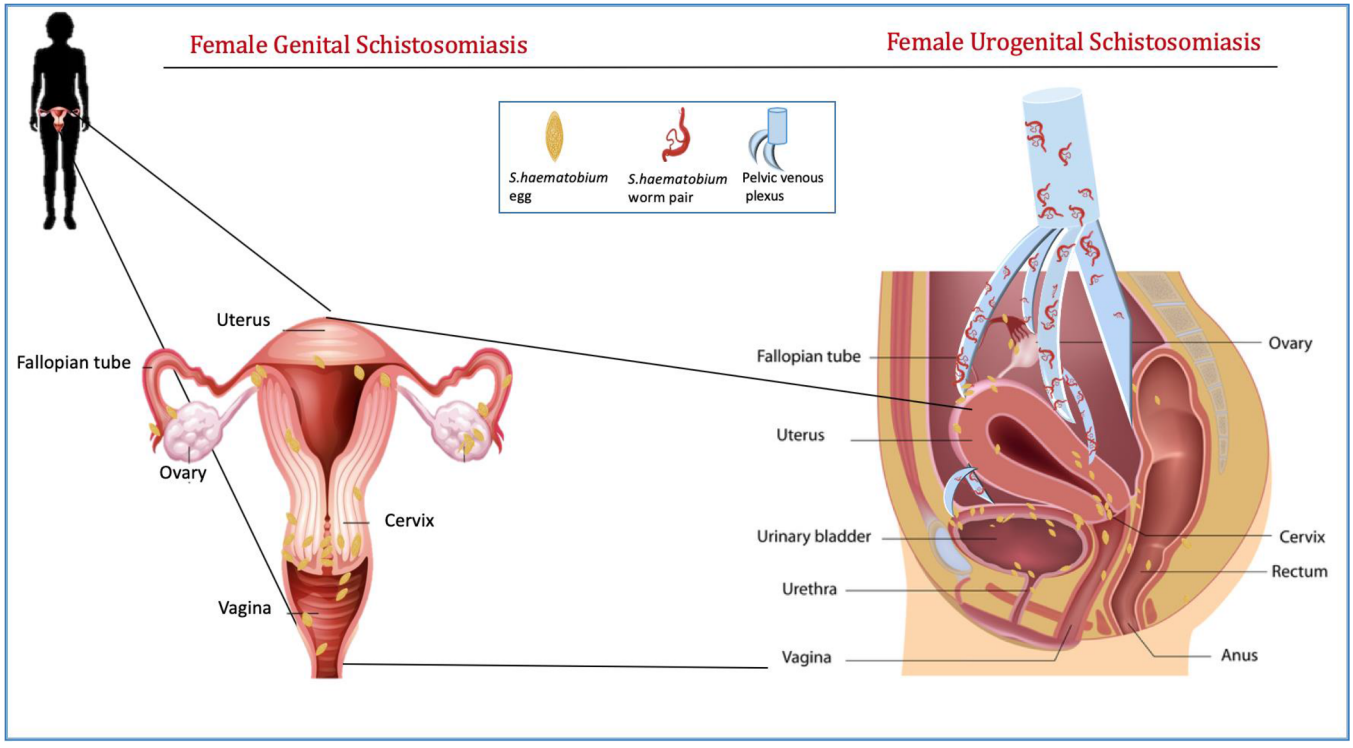

Figure 2. The anatomy of female genital and female urogenital schistosomiasis.

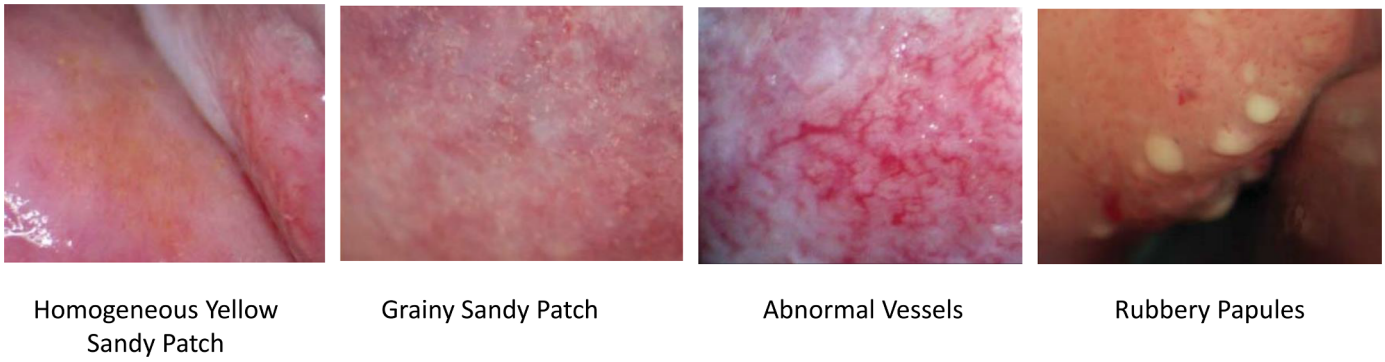

Figure 3. Visual findings suggestive of Female Genital Schistosomiasis, from World Health Organization (2015).

pathognomonic, symptoms included haematuria, dyspareunia, and post-coital bleeding (Kjetland et al., 1996). FGS has characteristic clinical findings: grainy sandy patches (representing ova near the mucosal surface) (Kjetland et al., 1996), homogenous yellow sandy patches (Kjetland et al., 2005), and rubbery papules (Figure 3). These lesions are associated with neovascularization (Kjetland et al., 1996; Kjetland et al., 2005) and contact bleeding (Kjetland et al., 1996; Kjetland et al., 2005). Common sites for egg visualization in adult females were the cervix (Kjetland et al., 2005), vagina (Kjetland et al., 2005), and vulva (Kjetland et al., 1996) while women under the age of 20 commonly presented with vulvar lesions (Helling-Giese et al., 1996; Swai et al., 2006). The difference in presentation may be related to anatomic, hormonal, and vascular changes associated with puberty (Helling-Giese et al., 1996).

3.4. Treatment of S. haematobium infection and FGS with praziquantel

Currently the World Health Organization (WHO) recommends praziquantel (PZQ) mass drug administration (MDA) to control *S. haematobium* disease and reduce morbidity. Data on the effect of PZQ on FGS lesion reversibility are scarce and subject to limitations. To date, three studies of adult women with FGS followed after treatment with PZQ have been conducted in Zimbabwe (n = 338), Tanzania (n = 33), and Malawi (n = 9). All three suggested that, following treatment with at least 40mg/kg of PZQ, a proportion of cervicovaginal lesions are irreversible with variation by lesion type, duration of follow-up, and praziquantel dose provided (Richter et al., 1996; Kjetland et al., 2006; Downs et al., 2013). Data from Zimbabwe suggested that women treated for schistosomiasis prior to the age of 20 had significantly lower prevalence of sandy patches and contact bleeding than untreated women (Kjetland et al., 2008). PZQ administration prior to the age of 21 has also been associated with lower rates of sub-fertility (Miller-Fellows et al., 2017). Thus, it is critical that schistosomiasis treatment occurs early, prior to the development of FGS lesions. Further well-designed clinical trials are required to investigate this question.

In addition to its role in schistosomiasis morbidity reduction, mathematical modelling suggests that PZQ treatment of women and school-aged children in *S. haematobium* high-risk communities could reduce HIV-1 prevalence (Gibson et al., 2010; Mushayabasa and Bhunu 2011; Ndeffo Mbah et al., 2013; Ndeffo Mbah et al., 2014). This finding highlights the need for further research into the use of PZQ for HIV-1 prevention. Other effective HIV-1 prevention methods include antiretroviral therapy and pre-exposure prophylaxis (PrEP). However, even with the use of linkage strategies, PrEP (Mugwanya, 2018) and ART uptake (Bor et al., 2018) and coverage are substantially less than 100% (Granich et al., 2015), and PrEP adherence in both men and women is imperfect (Koss et al., 2017). These limitations outline the need for a comprehensive approach to HIV-1 prevention, and further research is needed to evaluate praziquantel's role.

3.5. The impact of FGS on reproductive health

Due to its ability to affect both the upper and lower female reproductive tract, FGS has been implicated in poor reproductive health outcomes. However, data are mostly limited to pathology and case reports. Pathology reports described the presence of severe scarring (Swai et al., 2006) and inflammation (Wright et al., 1982; Swai et al., 2006) in the fallopian tubes related to the presence of *S. haematobium* eggs. Case reports described poor pregnancy outcomes in FGS, including stillbirth (Helling-Giese et al., 1996), ectopic pregnancy (Helling-Giese et al., 1996; Odubamowo et al., 2014) and spontaneous abortion (Helling-Giese et al., 1996; Friedman et al., 2007). The negative impact of FGS on reproductive health is most likely mediated through the histologic and mechanical effect of tissue destruction caused by schistosome eggs (Kjetland et al., 2010), but more research is urgently needed to understand the detrimental implications of FGS for a woman's sexual and reproductive health. Additional adverse outcomes attributed to *S. haematobium* infection in pregnancy include anemia, preterm labor, intrauterine growth restriction, and low birth weight (Friedman et al., 2007).

Infertility, a known consequence of upper reproductive tract pathology, is another reproductive health outcome that is associated with *S. haematobium* (Woodall and Kramer 2018). A 44% prevalence of subfertility (higher than worldwide averages of 8-12%) has been reported in women living in an area of high *S. haematobium* endemicity (60-85% prevalence in school-age children) (Miller-Fellows et al., 2017). FGS, defined in this study as the finding of *S. haematobium* ova in Papanicolau smears, was associated with primary infertility, defined as the inability to conceive after four years of regular sexual activity (OR 3.6; 1.0 – 12.0, p = 0.04) (Kjetland et al., 2010). The distribution of secondary infertility (infertility after giving birth to a child) overlaps substantially with the distribution of FGS in sub-Saharan Africa (Figure 1A and 1D).

4. Female Genital Schistosomiasis and HIV-1 co-infection

4.1. S. haematobium and HIV-1 – epidemiology and an ecological association

In addition to biologic plausibility supporting the association between an entity that causes cervicovaginal barrier disruption and HIV-1 acquisition, ecological associations have been reported between *S. haematobium* and HIV-1 (Mbah et al., 2013; Brodish and Singh 2016). The epidemiology of both schistosomiasis and HIV-1 may be influenced by interactions between the two infections, but individual studies have reported disparate conclusions regarding egg excretion, infection intensity, and association of schistosome infection with HIV-1. Ten studies that evaluated the association of non-genital schistosomiasis with HIV-1 met our inclusion criteria. Four studies with varied methodologies suggested an association between schistosome infection (genital

infection status was not reported) and HIV-1 (N'Zoukoudi-N'Doundou et al., 1995; Ndhlovu et al., 2007; Downs et al., 2012; Downs et al., 2017) albeit the association in two studies was only seen within sub-groups (Ndhlovu et al., 2007; Downs et al., 2017). Of note two of the studies found an association with HIV-1 evaluate *S. haematobium* (N'Zoukoudi-N'Doundou et al., 1995; Ndhlovu et al., 2007) and two studies measured CAA (Downs et al., 2012; Downs et al., 2017), which suggests active schistosome infection but cannot differentiate between *S. haematobium* or *S. mansoni* species. Conversely, six cross-sectional or case-control studies identified in our search evaluated the association between schistosome infection and HIV-1 did not show an association between schistosome and HIV-1 infection (Fontanet et al., 2000; Kallestrup et al., 2005; Mazigo et al., 2014; Sanya et al., 2015; Ssetaala et al., 2015; Downs et al., 2017), of note, none of these studies described FGS or *S. haematobium* infection in isolation. Meaningful comparison across studies was limited by variations in diagnostic methods and definitions of schistosome infection and many studies did not report analyses stratified by sex. In those that did, associations between schistosome infection and HIV-1 prevalence (Downs et al., 2012), increased acquisition (Downs et al., 2017), or transmission (Wall et al., 2018) were commonly (although not universally) seen in females but not in males. In females, cervicovaginal schistosome egg-containing tissue is exposed to semen containing HIV-1. The prostate and seminal vesicles are commonly affected by *S. haematobium* in men (Kayuni et al., 2019), internal structures that are not exposed during sexual contact. Authors finding a difference in the association between schistosomiasis and HIV-1 infection by sex hypothesize that this may be due to differential contact of egg-containing tissues in female versus male genital tissues during sexual contact (Downs et al., 2017).

4.2. FGS as a risk factor for the heterosexual acquisition of HIV-1

While the majority of studies examine the association between schistosome infection and HIV-1, our search identified only two studies that evaluate a definition of FGS that includes egg deposition in genital tissue, and its association with prevalent HIV-1 (Supplementary Table 1). Data from these two cross-sectional studies suggested that women with FGS have increased odds of having HIV-1 (Kjetland et al., 2006). One study performed in rural Zimbabwe, found that 41% (29/70) of women with FGS (diagnosed by *S. haematobium* eggs in Papanicolaou, wet smear, or genital biopsy) were HIV-1 positive, compared with 26% (96/375) in the egg negative group (OR 2.1, 95% CI 1.2-3.5; p = 0.008) (Kjetland et al., 2006). A study in Tanzania investigated HIV-1 prevalence among women with FUS (defined as either urinary *S. haematobium* egg excretion (16/23 participants) or egg detection in genital tissue (7/23 participants)), found that of the 23 women with FUS, 4 (17.4%) were HIV-1 infected compared with 23 (5.3%) of 434 women without FUS (OR 4.0, 95% CI 1.2–13.5) (Downs et al., 2011). This study was limited by small numbers, a low number of women with eggs detected in genital tissue, and the use of a broad FUS definition.

4.3. Schistosomiasis as a risk factor for HIV-1 incidence and transmission

To our knowledge, published data evaluating the association of FGS with HIV-1 incidence or transmission are lacking, although a small number of studies have evaluated the association of schistosome infection with HIV-1 (but genital infection status was not reported in these studies) (Downs et al., 2017; Wall et al., 2018). A retrospective analysis of a longitudinal cohort study of HIV-1 incidence in anti-retroviral therapy (ART) naïve, heterosexual HIV-1 serodiscordant couples in Zambia examined the association of baseline schistosome antibody status (as a proxy for previous or current infection) with incident HIV-1 acquisition and transmission (Wall et al., 2018). The presence of *S. haematobium* antibodies in 482 HIV-1 negative women was associated with increased risk of HIV-1 acquisition (Wall et al., 2018). However, limitations include the absence of information on the timing of the index partner's HIV-1 infection, the use of *Schistosoma* serology, which does not differentiate between past or present infection, and missing data on ART initiation. Research is needed to evaluate the association of FGS with HIV-1 incidence and transmission in the era of universal ART.

4.4. The intersection of FGS and host HIV-1 susceptibility

S. haematobium egg deposition in the female reproductive tract leads to a histological microenvironment that may enhance HIV-1 vulnerability. HIV-1 susceptibility is influenced by multiple factors but ultimately requires the presence of susceptible host cells (commonly in the vagina or cervix) (Kaul et al., 2015). HIV-1 fusion with and entry into susceptible target cells (T-lymphocytes, monocyte/macrophages or dendritic/Langerhans cells) requires the expression of the principal CD4 receptor and a chemokine co-receptor (CCR5 or CXCR4) (Kaul et al., 2015). These target cells and their chemokine co-receptors are found in the human genital tract (Kaul et al., 2015). HIV-1 susceptibility in the host is influenced by the overall number (Secor et al., 2003), density, and expression of the chemokine co-receptors on target cells that can be utilized by HIV-1 for cellular entry (Kaul et al., 2015). Certain CD4+ subsets are more susceptible to HIV-1 infection (McKinnon et al., 2011) and thus the availability, activation status, and phenotype of mucosal target cells influence HIV-1 susceptibility (Secor et al., 2003; McKinnon and Kaul 2012). In women with FGS, cervical tissue containing *S. haematobium* eggs has an increased density of CD4+ lymphocytes and macrophages compared to cervical tissue not containing eggs (Jourdan et al., 2011). On a histological level, cervical tissue containing *S. haematobium* eggs is also more vascularized (Jourdan et al., 2011) potentially allowing increased vascular access to HIV-1 target cells. Thus, the granuloma environment in *S. haematobium* infection theoretically encourages a milieu of cellular populations necessary to establish HIV-1 infection.

Compared to egg negative men, egg-positive *S. mansoni* infection in Kenyan males was associated with a higher density of CCR5 and CXCR4 co-receptors in the peripheral blood (Secor et al., 2003). Such data are not available in *S. haematobium* infection and are hypothesis-generating regarding the association between schistosome infection and HIV-1 status. Limited data exist regarding the influence of FGS on co-receptor expression. A small study conducted in KwaZulu-Natal, South Africa followed 14 women aged 15 – 23 years with FGS (defined by a suggestive clinical exam) for 8 months (Kleppa et al., 2014). Flow-cytometry was performed from peripheral blood and cervical cytobrush samples before and after praziquantel treatment. Compared to FGS negative women, participants with FGS had increased expression of the CCR5 co-receptor on plasma CD4 cells and vaginal CD14+ monocytes and increased frequencies of systemic monocytes (Kleppa et al., 2014). Increased frequencies of HIV-1 target cells and co-receptor expression are plausible mechanisms for increased HIV-1 vulnerability in women with FGS, but given the small sample size and the lack of histologic or laboratory diagnosis of FGS, additional research is needed.

5. Does FGS behave like an STI or bacterial vaginosis in heterosexual HIV-1 acquisition? Mechanistic hypotheses for the role of FGS in HIV-1 vulnerability

Although the association between FGS and HIV-1 has been hypothesized to be a direct result of FGS-associated lesions in the female genital tract (Feldmeier et al., 1994; Kjetland et al., 2005), the mechanism of the HIV-1 vulnerability has not been fully described. In ulcerative STIs (e.g. syphilis or herpes simplex virus (HSV)), damage to the protective cervicovaginal barrier is associated with increased risk of HIV-1 transmission (Gray et al., 2001) and acquisition (McKinnon and Kaul 2012). In this section we put forth several hypotheses (Figure 4) for how FGS may influence mechanisms associated with HIV-1 acquisition. In addition to causing barrier dysfunction, STIs, and perhaps also FGS, increase the risk of HIV-1 transmission through their contribution

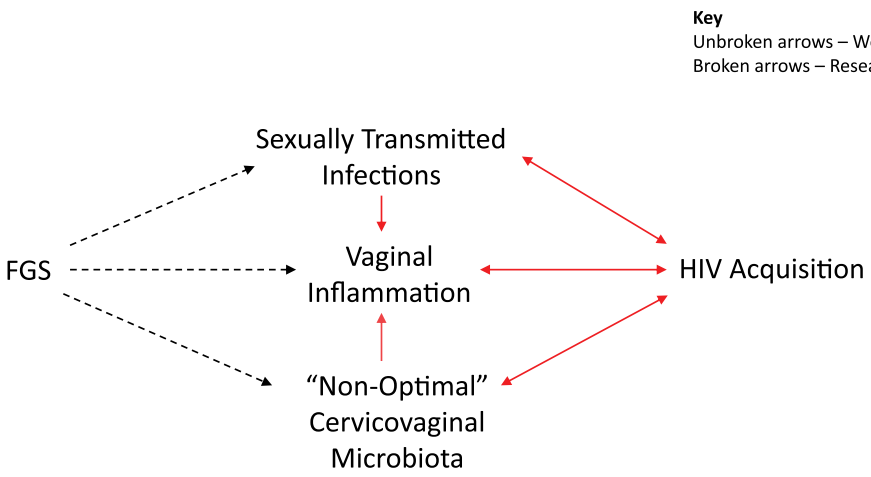

Figure 4. Conceptual pathway describing the potential contribution of FGS to vaginal inflammation and the association of FGS with sexually transmitted infection and "non-optimal" cervicovaginal microbiota (McKinnon et al., 2019).

to genital HIV-1 replication (Cohen et al., 1997) and HIV-1 acquisition through genital inflammation (Masson et al., 2014; Masson et al., 2015) and HIV-1 target cell recruitment (Masson et al., 2014).

5.1. Impaired cervicovaginal barrier function

In the female genital tract, the cervicovaginal immune defences are physical and immunological (Selhorst et al., 2017). An intact vaginal mucus layer, antimicrobial peptides, an acidic pH, and optimal vaginal microbiota combined with a preserved cervicovaginal epithelium provide an effective barrier, the first defence against HIV-1 acquisition (Selhorst et al., 2017). STIs are a classic example of how compromised cervicovaginal barriers increase susceptibility to HIV-1 acquisition. Like STIs, the characteristic lesions of FGS represent a breach in the intact cervicovaginal mucosal barrier and are hypothesized to be an entry point for HIV-1 (Feldmeier et al., 1995). At a population level, non-ulcerative STIs may be more important given their higher incidence and prevalence. And unlike many bacterial STIs which resolve with appropriate treatment, a proportion of FGS genital lesions may persist despite treatment with praziquantel (see section 3.4) (Downs et al., 2013). Further research is needed to evaluate if, similar to HSV ulcers, healed FGS lesions are associated with persistent localized inflammation, including HIV-1 target cells (Zhu et al., 2009).

5.2. The role of vaginal inflammation in HIV-1 acquisition. Could FGS contribute?

Inflammation and immune activation are central to HIV-1 pathogenesis and acquisition. Biological mechanisms that are influenced by vaginal inflammation and contribute to male-to-female heterosexual HIV-1 acquisition include mucosal target cell recruitment (McKinnon and Kaul 2012) and impaired cervicovaginal barrier function (Arnold et al., 2016). Initiation of the inflammatory cascade functions to recruit immunologic cellular mediators. In an escalating onward cascade, the presence of vaginal inflammation recruits the very cells needed to establish HIV-1 infection (Arnold et al., 2016). HIV-1 can infect T-lymphocytes, macrophages, and dendritic cells present in the cervicovaginal tissue (McKinnon and Kaul 2012). Elevated genital cytokines affect the expression of proteins associated with the cervicovaginal epithelium's integrity (Arnold et al., 2016). Compromise of this physical barrier occurs in FGS and provides a rich supply of activated and available subepithelial HIV-1 target cells.

S. haematobium infection has been associated with altered levels of systemic (Lyke et al., 2006; Erikstrup et al., 2008), seminal fluid (Leutscher et al., 2005), and vaginal cytokines (Dupnik et al., 2018). Raised vaginal concentrations of chemotactic (Morrison et al., 2014) and inflammatory cytokines are a risk factor for HIV-1 acquisition (Masson et al., 2015). In a study of 58 female HIV-1 seroconverters in South Africa, women with elevation of more than 5 out of 9 inflammatory cytokines had increased odds of HIV-1 acquisition (OR 3.2, 95% CI 1.3-7.9) (Masson et al., 2015). Mechanistically, the association has biological plausibility, as the production of inflammatory cytokines can influence expression of biomarkers of tissue remodelling and integrity (Arnold et al., 2016), modulate HIV-1 replication through transcription factor expression (Masson et al., 2014), or indirectly influence the differentiation, proliferation, and activation of HIV-1 target cells (Masson et al., 2014). Further research is needed to evaluate if FGS is associated with the genital inflammatory cytokines that are also associated with HIV-1 infection (Masson et al., 2015).

5.3. HIV-1 RNA concentrations, schistosomiasis, and HIV-1 transmission

Treatment of bacterial STI with antibiotics significantly decreases the concentration of HIV-1 RNA in seminal plasma (Cohen et al., 1997). Similarly, African men with genital schistosomiasis showed a decline in the concentration of HIV-1 RNA in seminal plasma after treatment with praziquantel (Midzi et al., 2017). Immunologically, egg-positive schistosome infection induces a strong Th2 bias (Pearce et al., 1991). Limited data suggest that HIV-1 replicates preferentially in activated CD4 Th0/Th2 cells (Maggi et al., 1994). Thus, there is immunologic plausibility to a hypothesis linking schistosomiasis with HIV-1 transmission. Practically, this may manifest as altered plasma and genital HIV-1 RNA concentrations among those who are dually infected with HIV-1 and schistosomiasis. Understanding the effect of schistosome infection on HIV-1 viral loads is critical given that schistosome infection in both women and men has been associated with HIV-1 transmission (Wall et al., 2018). However, the data regarding the association of schistosomiasis with elevated plasma viral loads are conflicting (Downs et al., 2017; Colombe et al., 2018; Bochner et al., 2019) and there may be a species-specific effect of *Schistosoma* on viral load (Bochner et al., 2019). In a study of HIV-1 seroconversion, participants with *S. haematobium* had higher set point plasma viral loads ($+0.33$ $\log_{10}$ copies, 95%CI -0.07-0.73, $p = 0.11$) compared to uninfected individuals, while participants with *S. mansoni* had lower set point HIV-1 viral loads (-0.34 $\log_{10}$ copies, 95% CI -0.58 to -0.09,$p = 0.007$) (Bochner et al., 2019). Recent longitudinal studies report both increased (Downs et al., 2017) and decreased (Colombe et al., 2018; Bochner et al., 2019) HIV-1 viral load set points in participants co-infected with HIV-1 and *S. mansoni.* Of note, FGS status was not evaluated in these studies (Downs et al., 2017; Colombe et al., 2018; Bochner et al., 2019). Differences in the results

highlight the need to adjust for ART use and duration of HIV-1 infection when evaluating HIV-1 viral load in the context of schistosomiasis (Downs et al., 2017; Colombe et al., 2018; Bochner et al., 2019).

An elevated genital HIV-1 viral load is a known risk factor for HIV-1 transmission (Baeten et al., 2011). An analysis of four prospective cohort studies in HIV negative persons in Kenya or Uganda who experienced HIV-1 seroconversion provided data regarding the association of schistosome infection with altered genital HIV-1 RNA concentrations. After adjusting for age, year of HIV-1 acquisition, and $\log_{10}$ plasma viral load, 8 women with *S. haematobium* infection who acquired HIV-1 were found to have lower cervical (ß = -0.59 [-1.11- -0.06], p = 0.029) and similar vaginal (ß = -0.09 (-0.65-0.46), p = 0.74) HIV-1 viral loads compared with participants who did not have schistosomiasis (Bochner et al., 2019). However, since the numbers were small, the estimates were not adjusted for multiple comparisons, and genital schistosomiasis status was not assessed, further research is needed to describe the association of FGS with genital HIV-1 RNA concentrations.

5.4. Vaginal microbiota in S. haematobium endemic countries and the risk of HIV-1 acquisition

Optimal vaginal microbiota are characterized by the presence of lactic acid and hydrogen-peroxide producing bacteria, often *Lactobacilli*. "Non-optimal" cervicovaginal microbiota (McKinnon et al., 2019) is not dominated by *Lactobacilli*. Bacterial vaginosis (BV) is one condition within this category (Cohen et al., 2012). BV involves the replacement of lactic acid producing bacteria by anaerobes, with bacterial species often clustering into distinct communities (McKinnon et al., 2019). Prevalence of BV is estimated to be high among women in sub-Saharan Africa (Cohen et al., 2012) and women of African descent. Although BV is seldom associated with clinically visible inflammation (McKinnon et al., 2019), it is consistently associated with elevated concentrations of pro-inflammatory immune proteins (Masson et al., 2014) and poor sexual and reproductive health outcomes, such as preterm delivery, miscarriage, pelvic inflammatory disease, and HIV-1 acquisition (Atashili et al., 2008). A meta-analysis of HIV-1 incidence studies reported that the presence of BV resulted in a 60% increased risk of HIV-1 acquisition (Atashili et al., 2008). Potential factors contributing to this increased risk include an increase in vaginal pH and upregulation of cytokines that promote HIV-1 replication (Masson et al., 2014). As a chronic and recurrent disruption of the genital tract environment, BV contributes a substantial population attributable fraction (PAF) of HIV-1 acquisition (Masese et al., 2015). Given the substantial risk for HIV-1 acquisition that BV poses in sub-Saharan Africa, it is critical to evaluate if FGS might also be associated with changes in the cervicovaginal microbiota. Since the cervicovaginal presence of FGS likely persists over time, FGS may, like BV, contribute a high PAF for HIV-1 acquisition.

Research on the role of the gut microbiota in persons with urinary *S. haematobium* suggested that the effect of urinary *S. haematobium* infection may be manifest in body compartments distinct from the location of egg deposition (Kay et al., 2015). The differential detection of microbes based on *Schistosoma* infection status extended into the urinary microbiota as well (Adebayo et al., 2017). That *S. haematobium* infection is associated with alteration in the gut and urinary microbiota lends plausibility to the hypothesis that FGS may alter the cervicovaginal microbiota. The composition of the vaginal microbiota played a role in HIV-1 acquisition, as demonstrated by a study of 236 South African women, 31 of whom acquired HIV-1 after a median 335 days of follow-up. This study illustrated that compared with *Lactobacillus crispatus* dominance, high-diversity vaginal bacterial communities with low *Lactobacillus* abundance was associated with HIV-1 acquisition (Gosmann et al., 2017), however FGS or schistosome infection was not evaluated. Women with anaerobic dominance were also found to have increased genital CD4+ T-cell numbers, concentrations of cytokines produced by activated CD4+ cells (Gosmann et al., 2017), and cytokines that have been associated with HIV-1 acquisition (Masson et al., 2015). Further research is needed to evaluate the potential mechanistic connection between the vaginal microbiota and FGS.

6. Female Genital Schistosomiasis – research priorities

This review highlights gaps in the literature and the need for revised research priorities that evaluate the mechanistic links between FGS and HIV-1 acquisition and transmission. Research regarding additional biological mechanisms for HIV-1 vulnerability in FGS is urgently needed and future research must explore the association of FGS with: 1) cervicovaginal HIV-1 concentrations 2) cytokines and chemokines associated with HIV-1 acquisition, and 3) vaginal microbial community composition.

7. Conclusion

FGS is a neglected gynaecological disease of poverty, affecting vulnerable women in sub-Saharan Africa. FGS may be an unmeasured regional risk factor that compounds biological and cultural covariates in a population at risk for HIV-1 acquisition and transmission. The clinical cervicovaginal findings of FGS demonstrate a breach in the physical epithelial barrier. This mechanical mucosal defect may allow greater access to the HIV-1 target cells in the sub-mucosa. However, the hypothesis that cervicovaginal barrier dysfunction in FGS is the sole mechanism linking FGS and HIV-1 acquisition is likely incomplete. The experience of STI and BV and their association with HIV-1 vulnerability includes consideration of additional co-factors: target cell recruitment, vaginal inflammation, and HIV-1 RNA concentrations. A more complete understanding of the association of FGS with HIV-1 will advance the research agenda on diagnostic, therapeutic and prevention strategies for this disabling disease, and its synergistic role in the HIV-1 epidemic.

Footnotes

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Amaya Bustinduy receives funding from the Wellcome Trust (Award 205954/Z/17/Z) and the Dowager Countess Eleanor Peel Trust (Award 246).

Dr E Webb and Professor R Hayes received salary funding from MRC Grant Reference MR/K012126/1, and Dr SC Francis received salary from MRC Grant Reference MR/N023692/1. These awards are jointly funded by the UK Medical Research Council (MRC), UK and the UK Department for International Development (DFID), UK under the MRC/DFID Concordat agreement and is also part of the EDCTP2 program supported by the European Union.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2020.105524.

References

Adebayo, A.S., Suryavanshi, M.V., Bhute, S., Agunloye, A.M., Isokpehi, R.D., Anumudu, C.I., Shouche, Y.S., 2017. "The microbiome in urogenital schistosomiasis and induced bladder pathologies. PLoS Negl Trop Dis 11 (8), e0005826.

Arnold, K.B., Burgener, A., Birse, K., Romas, L., Dunphy, L.J., Shahabi, K., Abou, M.,

Westmacott, G.R., McCorrister, S., Kwatampora, J., Nyanga, B., Kimani, J., Masson, L., Liebenberg, L.J., Abdool Karim, S.S., Passmore, J.A., Lauffenburger, D.A., Kaul, R., McKinnon, L.R., 2016. "Increased levels of inflammatory cytokines in the female reproductive tract are associated with altered expression of proteases, mucosal barrier proteins, and an influx of HIV-susceptible target cells. Mucosal Immunol 9 (1), 194–205.

Atashili, J., Poole, C., Ndumbe, P.M., Adimora, A.A., Smith, J.S., 2008. "Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS 22 (12), 1493–1501.

Baeten, J.M., Kahle, E., Lingappa, J.R., Coombs, R.W., Delany-Moretlwe, S., Nakku-Joloba, E., Mugo, N.R., Wald, A., Corey, L., Donnell, D., Campbell, M.S., Mullins, J.I., Celum, C., H. S. V. H. I. V. T. S. T. Partners in Prevention, 2011. Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. Sci Transl Med 3 (77), 77ra29.

Bochner, A.F., Secor, W.E., Baeten, J.M., van Dam, G.J., Szpiro, A.A., Njenga, S.M., Corstjens, P., Mackelprang, R.D., Mugo, N.R., Overbaugh, J., Celum, C., Mujugira, A., McClelland, R.S., Barnabas, R.V., 2019. "Schistosomiasis was not associated with higher HIV-1 plasma or genital set point viral loads among HIV seroconverters from four cohort studies. PLoS Negl Trop Dis 13 (11), e0007886.

Bor, J., Chiu, C., Ahmed, S., Katz, I., Fox, M.P., Rosen, S., Yapa, M., Tanser, F., Pillay, D., Barnighausen, T., 2018. "Failure to initiate HIV treatment in patients with high CD4 counts: evidence from demographic surveillance in rural South Africa. Trop Med Int Health 23 (2), 206–220.

Brodish, P.H., Singh, K., 2016. "Association Between Schistosoma haematobium Exposure and Human Immunodeficiency Virus Infection Among Females in Mozambique. Am J Trop Med Hyg 94 (5), 1040–1044.

Christinet, V., Lazdins-Helds, J.K., Stothard, J.R., Reinhard-Rupp, J., 2016. "Female genital schistosomiasis (FGS): from case reports to a call for concerted action against this neglected gynaecological disease. Int J Parasitol 46 (7), 395–404.

Cohen, C.R., Lingappa, J.R., Baeten, J.M., Ngayo, M.O., Spiegel, C.A., Hong, T., Donnell, D., Celum, C., Kapiga, S., Delany, S., Bukusi, E.A., 2012. "Bacterial vaginosis associated with increased risk of female-to-male HIV-1 transmission: a prospective cohort analysis among African couples. PLoS Med 9 (6), e1001251.

Cohen, M.S., Hoffman, I.F., Royce, R.A., Kazembe, P., Dyer, J.R., Daly, C.C., Zimba, D., Vernazza, P.L., Maida, M., Fiscus, S.A., Eron Jr., J.J., 1997. "Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. Lancet 349 (9069), 1868–1873.

Colley, D.G., Bustinduy, A.L., Secor, W.E., King, C.H., 2014. Human schistosomiasis. Lancet 383 (9936), 2253–2264.

Colombe, S., Corstjens, P., de Dood, C.J., Miyaye, D., Magawa, R.G., Mngara, J., Kalluvya, S.E., van Lieshout, L., van Dam, G.J., Downs, J.A., 2018. "HIV-1 Viral Loads Are Not Elevated in Individuals Co-infected With Schistosoma spp. After Adjustment for Duration of HIV-1 Infection. Front Immunol 9 (2005).

Downs, J.A., de Dood, C.J., Dee, H.E., McGeehan, M., Khan, H., Marenga, A., Adel, P.E., Faustine, E., Issarow, B., Kisanga, E.F., Kisigo, G.A., Ngahyolerwa, S., Zahoro, F., Miyaye, D., Magawa, R.G., Mngara, J., Lee, M.H., Corstjens, P., van Dam, G.J., Fitzgerald, D.W., 2017. "Schistosomiasis and Human Immunodeficiency Virus in Men in Tanzania. Am J Trop Med Hyg 96 (4), 856–862.

Downs, J.A., Dupnik, K.M., van Dam, G.J., Urassa, M., Lutonja, P., Kornelis, D., de Dood, C.J., Hoekstra, P., Kanjala, C., Isingo, R., Peck, R.N., Lee, M.H., Corstjens, P., Todd, J., Changalucha, J.M., Johnson Jr., W.D., Fitzgerald, D.W., 2017. "Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: A nested case-control study. PLoS Negl Trop Dis 11 (9), e0005968.

Downs, J.A., Kabangila, R., Verweij, J.J., Jaka, H., Peck, R.N., Kalluvya, S.E., Changalucha, J.M., Johnson, W.D., van Lieshout, L., Fitzgerald, D.W., 2013. "Detectable urogenital schistosome DNA and cervical abnormalities 6 months after single-dose praziquantel in women with Schistosoma haematobium infection. Trop Med Int Health 18 (9), 1090–1096.

Downs, J.A., Mguta, C., Kaatano, G.M., Mitchell, K.B., Bang, H., Simplice, H., Kalluvya, S.E., Changalucha, J.M., Johnson, W.D., Fitzgerald, D.W., 2011. "Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region. Am J Trop Med Hyg 84 (3), 364–369.

Downs, J.A., van Dam, G.J., Changalucha, J.M., Corstjens, P.L., Peck, R.N., de Dood, C.J., Bang, H., Andreasen, A., Kalluvya, S.E., van Lieshout, L., Johnson, W.D., Fitzgerald, D.W., 2012. "Association of Schistosomiasis and HIV infection in Tanzania. Am J Trop Med Hyg 87 (5), 868–873.

Dupnik, K.M., Lee, M.H., Mishra, P., Reust, M.J., Colombe, S., Haider, S.R., Yao, B., Vick, K., Zhang, T., Xiang, J., Miyaye, D., Magawa, R., Lyimo, E., Mukerebe, C., Mngara, J., Kalluvya, S.E., de Dood, C.J., van Dam, G.J., Corstjens, P., Downs, J.A., 2018. "Altered cervical mucosal gene expression and lower IL-15 levels in women with S. haematobium but not S. mansoni infection. J Infect Dis.

Erikstrup, C., Kallestrup, P., Zinyama-Gutsire, R.B., Gomo, E., van Dam, G.J., Deelder, A.M., Butterworth, A.E., Pedersen, B.K., Ostrowski, S.R., Gerstoft, J., Ullum, H., 2008. "Schistosomiasis and infection with human immunodeficiency virus 1 in rural Zimbabwe: systemic inflammation during co-infection and after treatment for schistosomiasis. Am J Trop Med Hyg 79 (3), 331–337.

Feldmeier, H., Krantz, I., Poggensee, G., 1994. "Female genital schistosomiasis as a risk-factor for the transmission of HIV. Int J STD AIDS 5 (5), 368–372.

Feldmeier, H., Krantz, I., Poggensee, G., 1995. "Female genital schistosomiasis: a neglected risk factor for the transmission of HIV? Trans R Soc Trop Med Hyg 89 (2), 237.

Fontanet, A.L., Woldemichael, T., Sahlu, T., van Dam, G.J., Messele, T., Rinke de Wit, T., Masho, W., Yeneneh, H., Coutinho, R.A., van Lieshout, L., 2000. "Epidemiology of HIV and Schistosoma mansoni infections among sugar-estate residents in Ethiopia. Ann Trop Med Parasitol 94 (2), 145–155..

Friedman, J.F., Mital, P., Kanzaria, H.K., Olds, G.R., Kurtis, J.D., 2007. "Schistosomiasis and pregnancy. Trends Parasitol 23 (4), 159–164.

Gasparyan, A.Y., Ayvazyan, L., Blackmore, H., Kitas, G.D., 2011. "Writing a narrative biomedical review: considerations for authors, peer reviewers, and editors. Rheumatol Int 31 (11), 1409–1417.

Gibson, L.R., Li, B., Remold, S.K., 2010. "Treating cofactors can reverse the expansion of a primary disease epidemic. BMC Infect Dis 10, 248.

Global Burden of Disease DALYs and Hale Collaborators, 2018. "Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392 (10159), 1859–1922.

Gosmann, C., Anahtar, M.N., Handley, S.A., Farcasanu, M., Abu-Ali, G., Bowman, B.A., Padavattan, N., Desai, C., Droit, L., Moodley, A., Dong, M., Chen, Y., Ismail, N., Ndung'u, T., Ghebremichael, M.S., Wesemann, D.R., Mitchell, C., Dong, K.L., Huttenhower, C., Walker, B.D., Virgin, H.W., Kwon, D.S., 2017. "Lactobacillus-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women. Immunity 46 (1), 29–37.

Granich, R., Gupta, S., Hersh, B., Williams, B., Montaner, J., Young, B., Zuniga, J.M., 2015. "Trends in AIDS Deaths, New Infections and ART Coverage in the Top 30 Countries with the Highest AIDS Mortality Burden; 1990-2013. PLoS One 10 (7), e0131353.

Gray, R.H., Wawer, M.J., Brookmeyer, R., Sewankambo, N.K., Serwadda, D., Wabwire-Mangen, F., Lutalo, T., Li, X., vanCott, T., Quinn, T.C., Rakai Project, T., 2001. "Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. Lancet 357 (9263), 1149–1153.

Hegertun, I.E., Sulheim Gundersen, K.M., Kleppa, E., Zulu, S.G., Gundersen, S.G., Taylor, M., Kvalsvig, J.D., Kjetland, E.F., 2013. S. haematobium as a common cause of genital morbidity in girls: a cross-sectional study of children in South Africa. PLoS Negl Trop Dis 7 (3), e2104.

Helling-Giese, G., Kjetland, E.F., Gundersen, S.G., Poggensee, G., Richter, J., Krantz, I., Feldmeier, H., 1996. "Schistosomiasis in women: manifestations in the upper reproductive tract. Acta Trop 62 (4), 225–238.

Jourdan, P.M., Holmen, S.D., Gundersen, S.G., Roald, B., Kjetland, E.F., 2011. "HIV target cells in Schistosoma haematobium-infected female genital mucosa. Am J Trop Med Hyg 85 (6), 1060–1064.

Jourdan, P.M., Roald, B., Poggensee, G., Gundersen, S.G., Kjetland, E.F., 2011. Increased vascularity in cervicovaginal mucosa with Schistosoma haematobium infection. PLoS Negl Trop Dis 5 (6), e1170.

Kallestrup, P., Zinyama, R., Gomo, E., Butterworth, A.E., van Dam, G.J., Erikstrup, C., Ullum, H., 2005. "Schistosomiasis and HIV-1 infection in rural Zimbabwe: implications of coinfection for excretion of eggs. J Infect Dis 191 (8), 1311–1320.

Kaul, R., Prodger, J., Joag, V., Shannon, B., Yegorov, S., Galiwango, R., McKinnon, L., 2015. "Inflammation and HIV Transmission in Sub-Saharan Africa. Curr HIV/AIDS Rep 12 (2), 216–222.

Kay, G.L., Millard, A., Sergeant, M.J., Midzi, N., Gwisai, R., Mduluza, T., Ivens, A., Nausch, N., Mutapi, F., Pallen, M., 2015. "Differences in the Faecal Microbiome in Schistosoma haematobium Infected Children vs. Uninfected Children. PLoS Negl Trop Dis 9 (6), e0003861.

Kayuni, S., Lampiao, F., Makaula, P., Juziwelo, L., Lacourse, E.J., Reinhard-Rupp, J., Leutscher, P.D.C., Stothard, J.R., 2019. "A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa. Parasite Epidemiol Control 4, e00077.

Kjetland, E.F., Kurewa, E.N., Mduluza, T., Midzi, N., Gomo, E., Friis, H., Gundersen, S.G., Ndhlovu, P.D., 2010. "The first community-based report on the effect of genital Schistosoma haematobium infection on female fertility. Fertil Steril 94 (4), 1551–1553.

Kjetland, E.F., Mduluza, T., Ndhlovu, P.D., Gomo, E., Gwanzura, L., Midzi, N., Mason, P.R., Friis, H., Gundersen, S.G., 2006. "Genital schistosomiasis in women: a clinical 12-month in vivo study following treatment with praziquantel. Trans R Soc Trop Med Hyg 100 (8), 740–752.

Kjetland, E.F., Ndhlovu, P.D., Gomo, E., Mduluza, T., Midzi, N., Gwanzura, L., Mason, P.R., Sandvik, L., Friis, H., Gundersen, S.G., 2006. "Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS 20 (4), 593–600.

Kjetland, E.F., Ndhlovu, P.D., Kurewa, E.N., Midzi, N., Gomo, E., Mduluza, T., Friis, H., Gundersen, S.G., 2008. "Prevention of gynecologic contact bleeding and genital sandy patches by childhood anti-schistosomal treatment. Am J Trop Med Hyg 79 (1), 79–83.

Kjetland, E.F., Ndhlovu, P.D., Mduluza, T., Gomo, E., Gwanzura, L., Mason, P.R., Kurewa, E.N., Midzi, N., Friis, H., Gundersen, S.G., 2005. "Simple clinical manifestations of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg 72 (3), 311–319.

Kjetland, E.F., Poggensee, G., Helling-Giese, G., Richter, J., Sjaastad, A., Chitsulo, L., Kumwenda, N., Gundersen, S.G., Krantz, I., Feldmeier, H., 1996. "Female genital schistosomiasis due to Schistosoma haematobium. Clinical and parasitological findings in women in rural Malawi. Acta Trop 62 (4), 239–255.

Kleppa, E., Ramsuran, V., Zulu, S., Karlsen, G.H., Bere, A., Passmore, J.A., Ndhlovu, P., Lillebo, K., Holmen, S.D., Onsrud, M., Gundersen, S.G., Taylor, M., Kjetland, E.F., Ndung'u, T., 2014. "Effect of female genital schistosomiasis and anti-schistosomal treatment on monocytes, CD4+ T-cells and CCR5 expression in the female genital tract. PLoS One 9 (6), e98593.

Koss, C.A., Bacchetti, P., Hillier, S.L., Livant, E., Horng, H., Mgodi, N., Mirembe, B.G., Gomez Feliciano, K., Horn, S., Liu, A.Y., Glidden, D.V., Grant, R.M., Benet, L.Z., Louie, A., van der Straten, A., Chirenje, Z.M., Marrazzo, J.M., Gandhi, M., 2017. Differences in Cumulative Exposure and Adherence to Tenofovir in the VOICE, iPrEx OLE, and PrEP Demo Studies as Determined via Hair Concentrations. AIDS Res Hum Retroviruses 33 (8), 778–783.

Lai, Y.S., Biedermann, P., Ekpo, U.F., Garba, A., Mathieu, E., Midzi, N., Mwinzi, P.,

N'Goran, E.K., Raso, G., Assare, R.K., Sacko, M., Schur, N., Talla, I., Tchuente, L.A., Toure, S., Winkler, M.S., Utzinger, J., Vounatsou, P., 2015. "Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: a systematic review and geostatistical analysis. Lancet Infect Dis 15 (8), 927–940.

Larsen, U., 2000. "Primary and secondary infertility in sub-Saharan Africa. Int J Epidemiol 29 (2), 285–291.

Leutscher, P., Ravaoalimalala, V.E., Raharisolo, C., Ramarokoto, C.E., Rasendramino, M., Raobelison, A., Vennervald, B., Esterre, P., Feldmeier, H., 1998. "Clinical findings in female genital schistosomiasis in Madagascar. Trop Med Int Health 3 (4), 327–332.

Leutscher, P.D., Pedersen, M., Raharisolo, C., Jensen, J.S., Hoffmann, S., Lisse, I., Ostrowski, S.R., Reimert, C.M., Mauclere, P., Ullum, H., 2005. "Increased prevalence of leukocytes and elevated cytokine levels in semen from Schistosoma haematobium-infected individuals. J Infect Dis 191 (10), 1639–1647.

Lyke, K.E., Dabo, A., Sangare, L., Arama, C., Daou, M., Diarra, I., Plowe, C.V., Doumbo, O.K., Sztein, M.B., 2006. "Effects of concomitant Schistosoma haematobium infection on the serum cytokine levels elicited by acute Plasmodium falciparum malaria infection in Malian children. Infect Immun 74 (10), 5718–5724.

Maggi, E., Mazzetti, M., Ravina, A., Annunziato, F., de Carli, M., Piccinni, M.P., Manetti, R., Carbonari, M., Pesce, A.M., del Prete, G., et al., 1994. "Ability of HIV to promote a TH1 to TH0 shift and to replicate preferentially in TH2 and TH0 cells. Science 265 (5169), 244–248..

Masese, L., Baeten, J.M., Richardson, B.A., Bukusi, E., John-Stewart, G., Graham, S.M., Shafi, J., Kiarie, J., Overbaugh, J., McClelland, R.S., 2015. "Changes in the contribution of genital tract infections to HIV acquisition among Kenyan high-risk women from 1993 to 2012. AIDS 29 (9), 1077–1085.

Masson, L., Mlisana, K., Little, F., Werner, L., Mkhize, N.N., Ronacher, K., Gamieldien, H., Williamson, C., McKinnon, L.R., Walzl, G., Abdool Karim, Q., Abdool Karim, S.S., Passmore, J.A., 2014. "Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study. Sex Transm Infect 90 (8), 580–587.

Masson, L., Passmore, J.A., Liebenberg, L.J., Werner, L., Baxter, C., Arnold, K.B., Williamson, C., Little, F., Mansoor, L.E., Naranbhai, V., Lauffenburger, D.A., Ronacher, K., Walzl, G., Garrett, N.J., Williams, B.L., Couto-Rodriguez, M., Hornig, M., Lipkin, W.I., Grobler, A., Abdool Karim, Q., Abdool Karim, S.S., 2015. "Genital inflammation and the risk of HIV acquisition in women. Clin Infect Dis 61 (2), 260–269.

Mazigo, H.D., Dunne, D.W., Wilson, S., Kinung'hi, S.M., Pinot de Moira, A., Jones, F.M., Morona, D., Nuwaha, F., 2014. "Co-infection with Schistosoma mansoni and Human Immunodeficiency Virus-1 (HIV-1) among residents of fishing villages of north-western Tanzania. Parasit Vectors 7, 587.

Mbah, M.L.N., Poolman, E.M., Drain, P.K., Coffee, M.P., van der Werf, M.J., Galvani, A.P., 2013. "HIV and Schistosoma haematobium prevalences correlate in sub-Saharan Africa. Trop Med Int Health 18 (10), 1174–1179.

McKinnon, L.R., Achilles, S.L., Bradshaw, C.S., Burgener, A., Crucitti, T., Fredricks, D.N., Jaspan, H.B., Kaul, R., Kaushic, C., Klatt, N., Kwon, D.S., Marrazzo, J.M., Masson, L., McClelland, R.S., Ravel, J., van de Wijgert, J., Vodstrcil, L.A., Tachedjian, G., 2019. "The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. AIDS Res Hum Retroviruses 35 (3), 219–228.

McKinnon, L.R., Kaul, R., 2012. "Quality and quantity: mucosal CD4+ T cells and HIV susceptibility. Curr Opin HIV AIDS 7 (2), 195–202.

McKinnon, L.R., Nyanga, B., Chege, D., Izulla, P., Kimani, M., Huibner, S., Gelmon, L., Block, K.E., Cicala, C., Anzala, A.O., Arthos, J., Kimani, J., Kaul, R., 2011. "Characterization of a human cervical CD4+ T cell subset coexpressing multiple markers of HIV susceptibility. J Immunol 187 (11), 6032–6042.

Midzi, N., Mduluza, T., Mudenge, B., Foldager, L., Leutscher, P.D.C., 2017. "Decrease in Seminal HIV-1 RNA Load After Praziquantel Treatment of Urogenital Schistosomiasis Coinfection in HIV-Positive Men-An Observational Study. Open Forum Infect Dis 4 (4), ofx199.

Miller-Fellows, S.C., Howard, L., Kramer, R., Hildebrand, V., Furin, J., Mutuku, F.M., Mukoko, D., Ivy, J.A., King, C.H., 2017. "Cross-sectional interview study of fertility, pregnancy, and urogenital schistosomiasis in coastal Kenya: Documented treatment in childhood is associated with reduced odds of subfertility among adult women. PLoS Negl Trop Dis 11 (11), e0006101.

Modjarrad, Kayvon, et al., 2010. Effect of treating co-infections on HIV-1 viral load: a systematic review. Lancet Infectious Diseases 10 (7), 455–463. https://doi.org/10.1016/S1473-3099(10)70093-1. PMID: 20610327.

Morrison, C., Fichorova, R.N., Mauck, C., Chen, P.L., Kwok, C., Chipato, T., Salata, R., Doncel, G.F., 2014. "Cervical inflammation and immunity associated with hormonal contraception, pregnancy, and HIV-1 seroconversion. J Acquir Immune Defic Syndr 66 (2), 109–117.

Mugwanya K Pintye J Kinuthia J et al "Uptake of PrEP within clinics providing integrated family planning and PrEP services: Results from a large implementation program in Kenya. Proceedings of the 22nd International AIDS Conference (AIDS 2018), Amsterdam, July 2018. Available at: http://programme.aids2018.org/Abstract/Abstract/7467 [accessed March 13, 2020]".

Mushayabasa, S., Bhunu, C.P., 2011. Modeling schistosomiasis and HIV/AIDS cody-namics. Comput Math Methods Med 2011, 846174.

N'Zoukoudi-N'Doundou, M.Y., Dirat, I., Akouala, J.J., Penchenier, L., Makuwa, M., Rey, J.L., 1995. "[Bilharziasis and human immunodeficiency virus infection in Congo]. Med Trop (Mars) 55 (3), 249–251.

Mbah, Ndeffo, L., M., Gilbert, J.A., Galvani, A.P., 2014. "Evaluating the potential impact of mass praziquantel administration for HIV prevention in Schistosoma haematobium high-risk communities. Epidemics 7, 22–27.

Ndeffo Mbah, M.L., Poolman, E.M., Drain, P.K., Coffee, M.P., van der Werf, M.J., Galvani, A.P., 2013. "HIV and Schistosoma haematobium prevalences correlate in sub-Saharan Africa. Trop Med Int Health 18 (10), 1174–1179.

Ndhlovu, P.D., Mduluza, T., Kjetland, E.F., Midzi, N., Nyanga, L., Gundersen, S.G., Friis, H., Gomo, E., 2007. "Prevalence of urinary schistosomiasis and HIV in females living in a rural community of Zimbabwe: does age matter? Trans R Soc Trop Med Hyg 101 (5), 433–438.

Odubamowo, K.H., Akinpelu, O.M., Lawal, O.O., Okolo, C.A., Odukogbe, A.A., Adekunle, A.O., 2014. "Bilateral tubal gestation associated with schistosomiasis in an african woman. Case Rep Obstet Gynecol 2014, 674514.

Pearce, E.J., Caspar, P., Grzych, J.M., Lewis, F.A., Sher, A., 1991. "Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. J Exp Med 173 (1), 159–166.

Poggensee, G., Kiwelu, I., Saria, M., Richter, J., Krantz, I., Feldmeier, H., 1998. "Schistosomiasis of the lower reproductive tract without egg excretion in urine. Am J Trop Med Hyg 59 (5), 782–783.

Richter, J., Poggensee, G., Kjetland, E.F., Helling-Giese, G., Chitsulo, L., Kumwenda, N., Gundersen, S.G., Deelder, A.M., Reimert, C.M., Haas, H., Krantz, I., Feldmeier, H., 1996. "Reversibility of lower reproductive tract abnormalities in women with Schistosoma haematobium infection after treatment with praziquantel–an interim report. Acta Trop 62 (4), 289–301.

Sanya, R.E., Muhangi, L., Nampijja, M., Nannozi, V., Nakawungu, P.K., Abayo, E., Webb, E.L., Elliott, A.M., s. t. La, V., 2015. "Schistosoma mansoni and HIV infection in a Ugandan population with high HIV and helminth prevalence. Trop Med Int Health 20 (9), 1201–1208.

Secor, W.E., Shah, A., Mwinzi, P.M., Ndenga, B.A., Watta, C.O., Karanja, D.M., 2003. "Increased density of human immunodeficiency virus type 1 coreceptors CCR5 and CXCR4 on the surfaces of CD4(+) T cells and monocytes of patients with Schistosoma mansoni infection. Infect Immun 71 (11), 6668–6671.

Selhorst, P., Masson, L., Ismail, S.D., Samsunder, N., Garrett, N., Mansoor, L.E., Abdool Karim, Q., Abdool Karim, S.S., Passmore, J.S., Williamson, C., 2017. "Cervicovaginal Inflammation Facilitates Acquisition of Less Infectious HIV Variants. Clin Infect Dis 64 (1), 79–82.

Ssetaala, A., Nakiyingi-Miiro, J., Asiki, G., Kyakuwa, N., Mpendo, J., Van Dam, G.J., Corstjens, P.L., Pala, P., Nielsen, L., Bont, J., Pantaleo, G., Kiwanuka, N., Kaleebu, P., Kamali, A., Elliott, A.M., 2015. "Schistosoma mansoni and HIV acquisition in fishing communities of Lake Victoria, Uganda: a nested case-control study. Trop Med Int Health 20 (9), 1190–1195.

Swai, B., Poggensee, G., Mtweve, S., Krantz, I., 2006. "Female genital schistosomiasis as an evidence of a neglected cause for reproductive ill-health: a retrospective histo-pathological study from Tanzania. BMC Infect Dis 6, 134.

UNAIDS 2018 "UN Joint Programme on HIV/AIDS AidsINFO 2018, available at: http://aidsinfo.unaids.org/ [accessed March 13, 2020]".

Wall, K.M., Kilembe, W., Vwalika, B., Dinh, C., Livingston, P., Lee, Y.M., Lakhi, S., Boeras, D., Naw, H.K., Brill, I., Chomba, E., Sharkey, T., Parker, R., Shutes, E., Tichacek, A., Secor, W.E., Allen, S., 2018. "Schistosomiasis is associated with incident HIV transmission and death in Zambia. PLoS Negl Trop Dis 12 (12), e0006902.

Woodall, P.A., Kramer, M.R., 2018. "Schistosomiasis and Infertility in East Africa. Am J Trop Med Hyg 98 (4), 1137–1144.

World Health Organization 2009 "Report of an informal working group meeting on urogenital schistosomiasis and HIV transmission. Geneva: World Health Organization; 2009. pp 1-35. Available at: https://www.who.int/schistosomiasis/resources/who_htm_ntd_pct_2010.5/en/ [accessed April 10, 2020]".

World Health Organization 2015 "Female genital schistosomiasis: a pocket atlas for clinical health-care professionals. World Health Organization. 2015. Available at, http://www.who.int/iris/handle/10665/180863 [accessed April 10, 2020]".

World Health Organization 2017 "Schistosomiasis, WHO reports substantial treatment progress for school-age children. 2017. Available at: https://www.who.int/neglected_diseases/news/WHO_schistosomiasis_reports_substantial_treatment_progress_sac/en/ [accessed April 10, 2020]".

Wright, E.D., Chiphangwi, J., Hutt, M.S., 1982. "Schistosomiasis of the female genital tract. A histopathological study of 176 cases from Malawi. Trans R Soc Trop Med Hyg 76 (6), 822–829.

Zhu, J., Hladik, F., Woodward, A., Klock, A., Peng, T., Johnston, C., Remington, M., Magaret, A., Koelle, D.M., Wald, A., Corey, L., 2009. "Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. Nat Med 15 (8), 886–892.

Studies Investigating Female Genital Schistosomiasis (FGS) and its Association with HIV-1 Infection in African Women

Reference	Location	Study Design	Sample Size	Age	FGS Defined	HIV Proportions	FGS Proportions	Method of Egg Detection	Association between FGS and HIV
Kjetland* 2006[1]	Zimbabwe	Cross - Sectional	479**	Median (range) 34 (20-49) years	Eggs in wet mount, biopsy, or pap smear	HIV (+) 28·0% (134/479)	FGS (+) 15·7% (70/445) FGS (–) 84·3% (375/445)	*S. haematobium* eggs in Papanicolau smear 6·0% (26/433); wet smear from the genital mucosa 40·0% (41/88); biopsy from genital tissue 57·7% (15/26); urine 39·4% (177/449). Any genital specimen 43·4% (70/445).	Of the 70 women with FGS, 29 (41·0%) were HIV infected, compared with 96 (26·0%) in the schistosome ova negative group. HIV was associated with FGS (OR 2·1; 95% CI 1·2 – 3·5, p=0·008).
Downs 2011[2]	Tanzania	Cross - Sectional	457	Median (IQR) 30 (24-35) years	Eggs in urine sample, cervical smear, or cervical biopsy	HIV (+) 5·9% (27/457)	FGS (+) 5·0% (23/457) FGS (–) 95·0% (434/457)	*S. haematobium* in urine 69·5% (16/23); *S. haematobium* in a genital specimen 26·1% (6/23); *S. mansoni* in cervical smear 4·3% (1/23)	Of the 23 women with FUS, 4 (17·4%) were HIV infected compared with 23 (5·3%) of 434 women without FGS. HIV was associated with FGS (OR 4·0; 95% CI 1·2 – 13·5, p=0·024).

* All women ages 20-49 years of age were invited to take part in this study, women were not required to consent to taking part in study procedures. Not all women consented to Papanicolau smears. Biopsies were only performed if malignancy was suspected. Wet smears from genital mucosa were performed only in the presence of mucosal bleeding.
** Data restricted to women who lived in the study area for more than 3 years

Abbreviations: FGS – female genital schistosomiasis, FUS – female urogenital schistosomiasis, HIV – human immunodeficiency virus

References:

1. Kjetland EF, Ndhlovu PD, Gomo E, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. *AIDS* 2006; **20**(4): 593–600.

2. Downs JA, Mguta C, Kaatano GM, et al. Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region. *Am J Trop Med Hyg* 2011; **84**(3): 364–9.

Studies Investigating the Association of Non-Genital Schistosomiasis with HIV-1 Infection in African Populations

Reference	Location	Study Design	Sample Size	Sex	Age (years)	Per participant method of Schistosome diagnosis *	Proportion with HIV-1 Infection	Proportion with Schistosome Infection	HIV Infection in participants with Schistosome Infection
Zoukoudi 1995[1]	Congo	Cross Sectional	895	Women 56·4% (505/ 895)	≥16	10 mL urine. If urine (–), then AWA-TCA serology (titre (+) at 1/160)	HIV (+) 5·5% (49/895)	Urine (+) *(S. haematobium)* 17·4% (156/895) Urine (–) Serology (+) 20·6% (185/895) Urine (+) or Serology (+) 38·1% (341/895) Urine (–) Serology (–) 61·9% (554/895)	In those with Urine (+) HIV-1 infection was 3.2% (5/156) compared with Urine (–) 5·9% (44/739), p=0·18 ** In those with Serology (+) HIV-1 infection was 3·8% (7/185) compared with Serology (–) 6·7%, (37/554), p=0·1** In those with Urine (+) and Serology (+) HIV-1 infection was 3·5%, (12/34) compared with Urine (–) and Serology (–), 6·7% (37/554), p=0·008
Fontanet 2000[2]	Ethiopia	Cross Sectional	1239	Women 28·2% (349/ 1239)	Mean (range) 30 (15-60)	One stool sample/ 1 KK smear (41mg stool) Urine CCA performed in a subset (n=287). (+) urine CCA >30 ng AWA-TCA/mL	HIV (+) 4·2% (52/1239)	Stool (+) *(S. mansoni)* 31·4% adjusted prevalence (358/1239) † Urine CCA (+) 49·8% (143/287)	Schistosome infection equally prevalent among HIV (–) 31·6% (348/1187) and HIV (+) 25·1% (10/52), (adjusted OR 1·01 (p=0·98))
Kallestrup 2005[3]	Zimbabwe	Cross Sectional	1545	Women 75·2% (1162/ 1545)	Median (IQR) 36·2 (25·8 - 46·4)	Fixed volume urine (3 consecutive days) One stool sample/MFE (1g stool)	HIV (+) 26·3% (407/1545)	Any Schistosome infection (by urine or stool) 43·4% (670/1545) No Schistosome infection 56·6% (875/1545)	Among 407 HIV-1 (+) participants, 222 (54·5%) were Schistosoma uninfected, 110 (27·0%) had *S. haematobium* infection only, 40 (9·8%) had *S. mansoni* infection only, and 35 (8·6%) were infected with both *S. haematobium* and *S. mansoni*

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								Urine (+) (*S. haematobium)* infection only 27·6% (426/1545) Stool (+) (*S. mansoni)* infection only 7·8% (121/1545) Urine and stool (+) *(S. haematobium* and *S. mansoni*) infection 8·0% (123/1545)	Corresponding figures among 1138 HIV-1 (–) participants were 653 (57·4%), 316 (27·8%), 81 (7·1%) and 88 (7·7%), respectively Overall p-value for association between HIV-1 and Schistosome infection was 0·31
Ndhlovu 2007[4]	Zimbabwe	Cross Sectional	544	Women 100·0%	Mean (range) 33.2 15-49	Urine sample (3 consecutive days)	HIV (+) 28·6% (156/544)	Urine (+) (*S. haematobium*) infection 39·7% (216/544)	Among 156 HIV-1 (+) participants, 84 (25·6%) were *S. haematobium* uninfected and 72 (33·3%) had *S. haematobium* infection (p=0.053), PR 1.30. In a sub-group of women ≥35 years of age, among 63 HIV-1 (+) participants, 30 (16.8%) were *S. haematobium* uninfected and 33 (37·5%) had *S. haematobium* infection (p<0.001), PR 2.23.
Downs 2012[5]	Tanzania	Cross Sectional	345	Women 100·0% (345/ 345)	Median (IQR) 30 (24-35)	Phlebotomy (serum CAA detection)	HIV (+) 6·1% (21/345)	CAA (+) 53·6% (185/345) CAA (–) 46·4% (160/345)	Among CAA (+) women, HIV-1 infection was 9·2% (17/185) compared with 2·5% (4/160) in CAA (–) (adjusted OR = 6·2 [95% CI = 1·7-22·9], p=0·006)
Mazigo 2014[6]	Tanzania	Cross Sectional	1785	Women 52·9% (945/ 1785)	Mean (SD) 35·6 (9·74)	One stool sample/KK – 4 smears (47·1 mg)	HIV-1 (+) 7·0% (125/1785)	Stool (+) *(S. mansoni)* 47·9% (854/1785)	In HIV-1 (–) the prevalence of *S. mansoni* (+) was 48·1% (804/1660) compared with HIV-1 (+) individuals 39·5% (50/125) (aPR 1·01, [95% CI 0·84-1·21], p=0·93)
Sanya 2015[7] TMIH	Uganda	Cross Sectional	1412	Women 42·0% (593/ 1412)	Mean (SD) 30·3 (9·5)	Single stool sample – 2 KK smears Urine CCA performed in a subset (n=650)	HIV-1 (+) 17·3% (244/1412)	Stool (+) (*S. mansoni)* 57·2% (719/1257) CAA (+) 73·5% (478/650)	In Stool (+) 16·1% (116/719) were HIV-1 (+) compared with 17·8% (96/538) in Stool (–) (aOR=1·04; [95% CI 0·74 - 1·47], p=0·81) In CAA (+) 20·7% (99/478) were HIV-1 (+) compared with 18·0% (31/172) who

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									were CAA (–) (aOR=1·53; [95% CI 0·78-3·00], p=0·19)
Ssetaala 2015[8]	Uganda	Case-control nested within a cohort study	200	Women 44·0% (88/200)	Median (IQR) 25 (22-30)	3 stool samples – KK or FME Stored serum for CAA @ 6, 12, 18 months. (+) serum CAA ≥ 32 pg/mL	HIV-1 (+) 25·0% (50/200) ††	CAA (+) at cohort enrollment 51% (102/200) CAA (+) at other timepoints: 6 months - 48·0% (96/200) 12 months - 52·0% (104/200) 18 months - 52·0% (104/200)	HIV-1 infection 49·0% (24/50) of cases and 52·0% (78/150) of controls had *S. mansoni* (CAA (+) infection prior to HIV-1 seroconversion (or a similar study visit for controls) (aOR 1·23 [95% CI 0·3-5·7], p=0·79))
Downs 2017[9]	Tanzania	Cross Sectional	674	Men 100·0% (674/674)	Median (IQR) 34 (25-42)	10mL urine 1 stool sample – 5 KK slides (41.7mg/slide) Phlebotomy for CAA (positive ≥30 pg/mL) <u>Definitions:</u> S. *mansoni* infection: eggs in stool or CAA (+) with no *S. haematobium* eggs in urine *S. haematobium* infection: eggs in urine or CAA (+) with no *S. mansoni* eggs in stool	HIV-1 (+) 5·6% (38/674)	CAA (+) or Egg (+) 63·6% (429/674) *S. mansoni* (Stool (+) or serum CAA (+) with Urine (–)) 49·9% (336/674) *S. haematobium* (Urine (+) or serum CAA (+) with Stool (–)) 14·1% (95/674) *S. mansoni* Stool (+) 34·4% (232/674) *S. haematobium* Urine (+) 5·5% (37/674)	In HIV-1 (+), 68·4% (26/38) had any schistosome infection (CAA (+) or egg (+)) compared with 63·4% (403/636) among HIV-1 (–) (OR=1·3 [0·6-2·5, p=0·53]) In HIV (+), 5·3% (2/38) were *S. haematobium* egg (+) compared with 5·5% (35/636) among HIV-1 (–) (OR not calculated due to small numbers, p=1·0) In HIV (+), 29·0% (11/38) were *S. mansoni* egg (+) compared with 34·8% (221/636) among HIV-1 (–) (OR=0·8; [95% CI 0·4- 1·9], p=0·63)
Downs 2017[10]	Tanzania	Case-control nested	338	Cases: Women	Cases: median	Capillary blood (CAA from dried	HIV-1 (+) 21·6% (73/338) ††	Total CAA (+) 34·0% (115/338)	Overall: 38% (28/73) HIV-1 seroconverters were CAA (+) compared

		within a cohort survey		61·6% (45/73) Controls: Women 61·1% (162/ 265)	(IQR) 35 (25-43) Controls: median (IQR) 34 (25-44)	blood spots before & after HIV-1 seroconversion) #		Women CAA (+) 32·8% (68/207) Men CAA (+) 35·8% (47/131)	to 32.0% (87/265) HIV-1 (-) controls (p=0.43) Women: 44·0% (20/45) HIV-1 seroconverters were CAA (+) compared to 30·0% (48/162) HIV-1 (–) controls (OR=2·8 [95% CI, 1·2-6·6], p=0·01) Men: 29·0% (8/28) HIV-1 seroconverters were CAA (+) compared to 38·0% (39/103) HIV-1 (–) controls (OR 0·7 [0·3-1·8], p=0·42)

*samples collected are per participant
**No difference when analyzed by sex (p – value not given)
† Stool (+) prevalence was adjusted for the survey design, but for Urine (+), unadjusted prevalence is presented since no adjusted estimates were reported
†† Participants were selected as cases and controls on the basis of their HIV-1 status
# Threshold for positivity not reported

Abbreviations: aOR – (adjusted odds ratio), AWA-TCA – trichloroacetic-acid-soluble fraction of the adult worm antigen (contains approximately 3% CCA, CCA – circulating cathodic antigen, FGS – female genital schistosomiasis, FUS – female urogenital schistosomiasis, HIV – human immunodeficiency virus, KK – Kato Katz, MFE – modified formol-ether concentration, OR – odds ratio, PR – prevalence ratio

References:

1. N'Zoukoudi-N'Doundou MY, Dirat I, Akouala JJ, Penchenier L, Makuwa M, Rey JL. [Bilharziasis and human immunodeficiency virus infection in Congo]. *Med Trop (Mars)* 1995; **55**(3): 249-51.
2. Fontanet AL, Woldemichael T, Sahlu T, et al. Epidemiology of HIV and Schistosoma mansoni infections among sugar-estate residents in Ethiopia. *Annals of tropical medicine and parasitology* 2000; **94**(2): 145-55.
3. Kallestrup P, Zinyama R, Gomo E, et al. Schistosomiasis and HIV-1 infection in rural Zimbabwe: implications of coinfection for excretion of eggs. *The Journal of infectious diseases* 2005; **191**(8): 1311-20.
4. Ndhlovu PD, Mduluza T, Kjetland EF, et al. Prevalence of urinary schistosomiasis and HIV in females living in a rural community of Zimbabwe: does age matter? *Trans R Soc Trop Med Hyg* 2007; **101**(5): 433-8.
5. Downs JA, van Dam GJ, Changalucha JM, et al. Association of Schistosomiasis and HIV infection in Tanzania. *Am J Trop Med Hyg* 2012; **87**(5): 868-73.
6. Mazigo HD, Dunne DW, Wilson S, et al. Co-infection with Schistosoma mansoni and Human Immunodeficiency Virus-1 (HIV-1) among residents of fishing villages of north-western Tanzania. *Parasites & vectors* 2014; **7**: 587.

7. Sanya RE, Muhangi L, Nampijja M, et al. Schistosoma mansoni and HIV infection in a Ugandan population with high HIV and helminth prevalence. *Trop Med Int Health* 2015; **20**(9): 1201-8.
8. Ssetaala A, Nakiyingi-Miiro J, Asiki G, et al. Schistosoma mansoni and HIV acquisition in fishing communities of Lake Victoria, Uganda: a nested case-control study. *Trop Med Int Health* 2015; **20**(9): 1190-5.
9. Downs JA, de Dood CJ, Dee HE, et al. Schistosomiasis and Human Immunodeficiency Virus in Men in Tanzania. *Am J Trop Med Hyg* 2017; **96**(4): 856-62.
10. Downs JA, Dupnik KM, van Dam GJ, et al. Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: A nested case-control study. *PLoS Negl Trop Dis* 2017; **11**(9): e0005968.

Chapter 3 – PhD thesis content and the candidate's contribution

Chapters 1 and *2* define FGS, describe the burden of FGS in sub-Saharan Africa, discuss the pathophysiology of HIV-1 acquisition, examine the role of the cervicovaginal environment, and identify knowledge gaps. Additionally, *Chapter 2* describes potential mechanisms for HIV-1 vulnerability in FGS. This PhD thesis aims to address knowledge gaps in our understanding of HIV-1 incidence and the cervicovaginal environment in women with "PCR-defined FGS". This chapter describes an overview of the PhD thesis, presents the primary aim, key thesis objectives and hypotheses, outlines the contents of each chapter and describes the candidate's role in this work.

3.1 Thesis aim

The overall aim of this PhD thesis was to describe the cervicovaginal microbiota (including STIs) and the cervicovaginal immune environment, focusing on cytokines and chemokines, in Zambian women with and without "PCR-defined FGS" and to explore the association of "PCR-defined FGS" with HIV-1 incidence.

3.2 Thesis objectives and hypotheses

1. **FGS and the Vaginal Microbiota:** To describe the association of "PCR-defined FGS" with the concentration of vaginal *Lactobacilli*, as well as describing the presence and concentrations of other key vaginal microbiota in women with and without "PCR-defined FGS".
    - Hypothesis: FGS will be associated with cervicovaginal microbiota in their "non-optimal" state.
2. **FGS and Sexually Transmitted Infections:** To describe the association of PCR-defined FGS with sexually transmitted infections.
    - Hypothesis: FGS will be associated with PCR-defined STIs.

3. **FGS and the Cervicovaginal Immune Environment:** To assess the association between FGS and the vaginal microenvironment by comparing:
    a. the concentration of selected HIV-acquisition-associated inflammatory markers in the cervicovaginal fluid of Zambian women with and without FGS.
    b. the cervicovaginal inflammatory signature in women with and without FGS with a focus on the t-helper type 1 (Th1) and t-helper type 2 (Th2) immune responses.
    - Hypothesis: FGS acts as a risk factor for HIV-1 acquisition by promoting elevated concentrations of chemokines associated with HIV-1 acquisition and contributing to genital inflammation.

4. **FGS and HIV-1 Incidence:** To compare HIV-1 incidence rates in Zambian women with and without FGS.
    - Hypothesis: FGS is associated with incident HIV-1 infection in Zambian women.

3.3 Thesis design

This PhD thesis is comprised of the results from three sub-studies using specimens and data collected in a cross-sectional study conducted in Livingstone, Zambia as well as a narrative review. The narrative review assesses literature published regarding the association of schistosomiasis and FGS with HIV-1. This PhD thesis uses data and specimens collected in the cross-sectional BILHIV study (Appendix 1). The primary aim of the BILHIV study was to validate home-based genital self-testing for *Schistosoma* DNA by PCR versus clinic-based testing (Appendix 1). I utilized specimens collected in the BILHIV study to perform two independent studies, described in this PhD thesis. The first study utilized quantitative PCR to characterize the cervicovaginal microbiota in Zambian women with and without FGS. The second study evaluated cytokine and chemokine concentrations in women with and without FGS (Figure 3.1). One of the BILHIV study objectives was to evaluate HIV-1 incidence in women with and without FGS and I further developed the project for this PhD thesis.

Figure 3.1 – PhD thesis components in relationship to the BILHIV study

3.4 Thesis structure

Chapter 1: The initial chapter presents background on FGS and introduces the knowledge gaps this PhD thesis seeks to fill.

Chapter 2: This chapter presents a published manuscript entitled "Beyond the barrier: Female genital schistosomiasis as a potential risk factor for HIV-1 acquisition". The narrative review is "Research Paper 1" and further expands on concepts introduced in *Chapter 1*, including FGS morbidity, clinical presentation, treatment, and reproductive health impact. The narrative review also provides a summary of the epidemiology of HIV-1 and schistosomiasis co-infection and puts forward hypotheses for how FGS may influence mechanisms associated with HIV-1 acquisition, transmission, and host HIV-1 susceptibility. *Chapter 2* also proposes a mechanistic hypothesis for the role of FGS in HIV-1 vulnerability, discussing barrier function, vaginal inflammation, HIV-1 RNA concentrations and the cervicovaginal microbiota.

Chapter 3: This chapter summarizes the PhD thesis objectives, hypotheses and introduces the thesis structure and the candidate's role.

Chapter 4: This chapter includes the methods used to address PhD thesis objectives outlined above (Section 3.2).

Chapter 5: This chapter contains "Research Paper 2" and includes a submitted manuscript entitled "Associations of female genital schistosomiasis with the cervicovaginal microbiota and reproductive tract infections in Zambian women". This manuscript corresponds with PhD thesis objectives 1 and 2 (Section 3.2).

Chapter 6: This chapter contains "Research Paper 3" and includes a published manuscript entitled "Cervicovaginal immune activation in Zambian women with female genital schistosomiasis". This manuscript corresponds with PhD thesis objective 3 (Section 3.2).

Chapter 7: This chapter contains "Research Paper 4" and includes a submitted manuscript entitled "Female genital schistosomiasis and HIV-1 incidence in Zambian women: a retrospective cohort study". This manuscript corresponds with PhD thesis objective 4 (Section 3.2).

Chapter 8: This chapter summarizes the findings from *Chapters 5-7* with a discussion of their implications in relationship to the current literature, study strengths and limitations, and priorities for future work.

This PhD thesis follows the guidance for a "research paper" style thesis with the presentation of four original manuscripts. Rather than presenting chapters in the temporal order of publication, the chapters are organized to create a cohesive flow of ideas.

Appendices contain relevant publications not included in this PhD thesis.

3.5 The candidate's role in the BILHIV study

January – August 2017: As a volunteer, I was responsible for developing study documents for the parent study, the BILHIV study. I developed the BILHIV study protocol, information sheets, consent forms, study questionnaires, clinic review forms and standard operating procedures together with my supervisor. I formatted the study questionnaires and uploaded them to the study tablet computers. Participant data from the study questionnaires was uploaded directly into the LSHTM server, comprising the study database. I was responsible for drafting the ethical approval for both London School of Hygiene and Tropical Medicine (LSHTM) and Zambia and was also responsible for renewals and amendments. I applied for tablet computers and maintained these device subscriptions through the LSHTM Device Library. I also established and maintained Open Data Kit (ODK) server access, as hosted at LSHTM, and was one of the server data administrators. I also sourced equipment and supplies to be used in the BILHIV study.

September 2017: I officially entered the PhD program at LSHTM

January - August 2018: During the BILHIV Study enrolment, I was one of two study managers and assisted with study oversight, performing administrative duties related to the study, data management and cleaning. Additionally, I analysed the data and contributed substantially to the manuscripts for two peer-reviewed publications not included in this PhD thesis (Appendix 1 and Appendix 2).

September 2018 – Present: I was the data manager for the BILHIV study and was responsible for cleaning and analysing BILHIV study data.

3.6 The candidate's independent PhD work

Research paper 1 – "Beyond the barrier: female genital schistosomiasis as a potential risk factor for HIV-1 acquisition"

- I performed the literature search, retrieved relevant studies, and extracted data from relevant studies together with my supervisor.
- I created data tables together with my supervisor.
- I assembled three of the four figures in the manuscript.

Research paper 2 – "Associations of female genital schistosomiasis with the cervicovaginal microbiota and reproductive tract infections in Zambian women"

- I selected the microbiota and STIs included in this study with the support of my supervisors and collaborators.
- I learned DNA extraction and PCR methods with the support of collaborators.
- I wrote the data analysis plan and performed the statistical analysis with support from my supervisor.

Research paper 3 – "Cervicovaginal immune activation in Zambian women with female genital schistosomiasis"

- I selected the cytokines and chemokines included in this study with the support of my supervisors and collaborators.
- Together with my supervisors, I applied for funding and received a small grant to cover the costs of the bead-based assays.
- I learned bead-based assay techniques at the LSHTM prior to the experiments. I then performed the bead-based assays together with technician Catriona Patterson at LSHTM.
- I wrote the data analysis plan and performed the statistical analysis with support from my supervisor.

Research paper 4 – "Female genital schistosomiasis and HIV-1 incidence in Zambian women: a retrospective cohort study".

- I applied to Statistical Center for HIV/AIDS Research and Prevention (SCHARP) to obtain access to the HPTN 071 (PopART) Population Cohort HIV-1 data.
- I wrote the data analysis plan, merged the SCHARP database together with the BILHIV database, evaluated data completeness, and performed the statistical analysis with support from my supervisor.

For all four research papers, I wrote the first draft of the manuscript and revised the manuscripts according to supervisor and co-author input. I submitted the final version of each manuscript for peer review and, together with co-authors, responded to comments from peer reviewers.

Chapter 4 – Methods

4.1 The HPTN 071 (PopART) trial

HPTN 071 (PopART) is a trial to measure the impact of an HIV combination prevention package, including universal HIV-1 test and treat, on HIV-1 incidence [105]. Twenty-one communities in Zambia and South Africa were selected and were grouped into triplets based on location and HIV-1 prevalence. The three communities in each triplet were randomly assigned to one of three trial arms: arm A (combination prevention plus universal anti-retroviral therapy [ART]), arm B (combination prevention plus ART provided based on local guidelines), and arm C (standard care). The combination prevention package was provided by trained community workers and included HIV-1 counselling and testing with linkage to HIV-1 care for ART initiation as well as adherence support. The community workers also screened participants for tuberculosis, referred male participants for circumcision and connected pregnant participants to clinical services for the prevention of mother-to-child-transmission [105]. The primary outcome for HPTN 071 (PopART) was HIV-1 incidence during the three-year intervention period, measured in a Population Cohort (PC). The PC included one randomly selected adult 18 to 44 years of age from a random sample of households in each community [105].

4.2 The BILHIV study

4.2.1 BILHIV study site and subjects

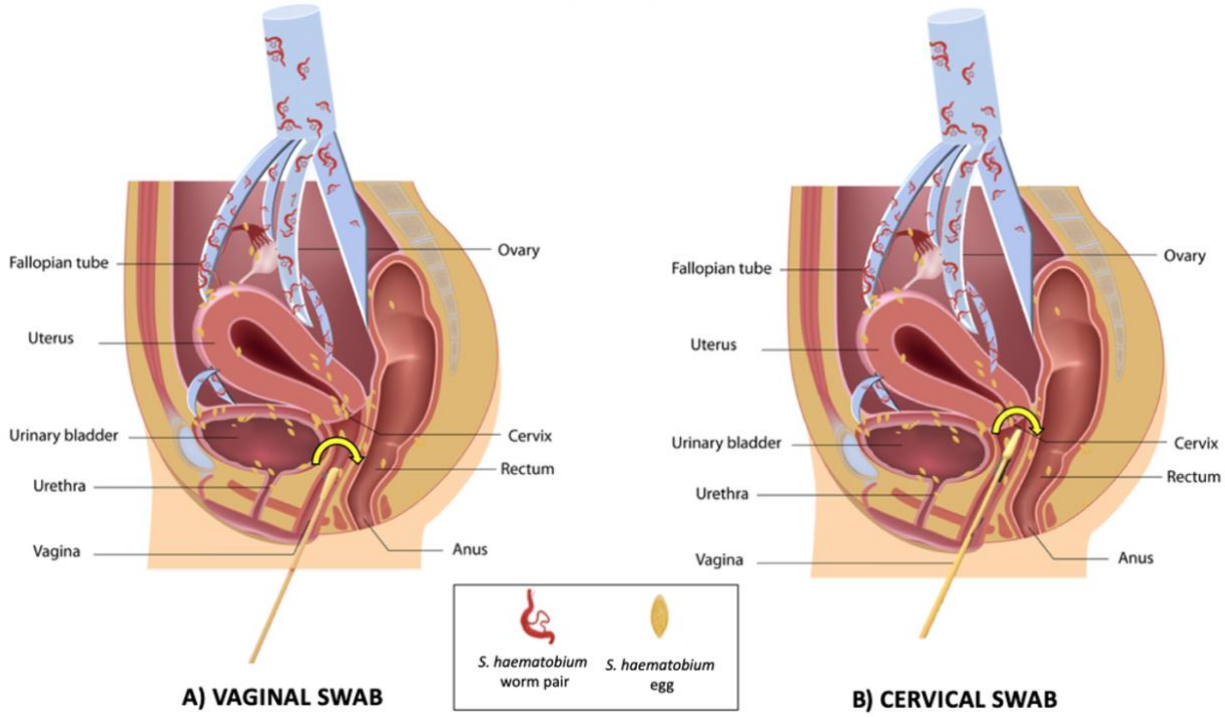
Between January and August 2018, participants from the PC of HPTN 071 (PopART), were recruited to participate in the cross-sectional bilharzia and HIV-1 (BILHIV) study. Women were eligible to participate if they were 18-31 years old, non-pregnant, sexually active, and resident in one of the two communities (designated Community-A and Community-B) that participated in HPTN 071 (PopART) in Livingstone, Zambia. Central Livingstone is located within 5-10 kilometres of the Zambezi River, with a tributary flowing in close proximity to Community A. BILHIV study participants were selected as a consecutive sample from the HPTN 071 (PopART) Population Cohort who completed their three-year PC study visit prior to or during BILHIV study enrolment (January – August 2018). A 2013 survey of school aged children in Livingstone reported prevalence ranges for urinary *S. haematobium* infection between 3.3% and 73.3% (median 15.0%, mean 23.3%) [106].

4.2.2 BILHIV study home-based sample collection

Trained community workers provided home visits to women who gave an "expression of interest" in the BILHIV study at the HPTN 071 (PopART) PC 36-month visit. The home visit included assessment of eligibility, a questionnaire, genital self-sampling (cervical and vaginal), and a single urine specimen. Trained field workers provided study information in the participant's preferred language (English, Tonga, Bemba, Lozi, or Nyanja). Following written informed consent, a questionnaire containing questions regarding demographics, water contact, sexual behaviour, and genital symptoms, was administered. There were no restrictions on the timing of urine sample self-collection, and 69.5% (419/603) were performed between 9:00 and 14:00. The community worker provided participating women with instructions for urine collection and cervical and vaginal self-sampling. Participants were instructed to hold a 6-inch PrimeSwab (Longhorn Diagnostics, Texas, USA) at the 2 3/8-inch score mark, insert the swab vaginally until their fingers touched the labia minora, and rotate the swab against the vaginal walls (minimum 15 repetitions) (Figure 4.1). Similarly, for the cervical swab, participants were instructed to hold a 6 3/4-inch flocked swab (Miraclean, Shenzen, China) with a quadrilateral kite-shaped tip at the non-flocked end, insert the swab until their fingers touched the labia minora and/or encountered resistance, and rotate the swab against the place of resistance (minimum 15 repetitions). Each flocked swab head was placed in individual screw cap microtubes (STARLAB, Hamburg, Germany) by the participant after the swab shaft was broken. Both swab specimens and urine were placed in cool boxes for transportation to the laboratory at -80°C for further processing.

Women with evidence of active schistosome infection, defined by any positive urine examination (microscopy, CAA or PCR), or women with clinical evidence of FGS as determined by the midwife's clinical examination [27], were treated free of charge with 40 mg/kg praziquantel, either at the clinic visit, or via community workers.

Figure 4.1 – Genital self-sampling technique utilized in the BILHIV study



4.2.3 BILHIV study clinic-based sample collection

Enrolled women who were not currently menstruating were invited to attend Livingstone Central Hospital cervical cancer screening clinic, where one of two trained midwives performed cervicovaginal lavage (CVL), which was used for *Schistosoma* PCR. After speculum insertion, a bulb syringe was used to flush normal saline (10 mL) continuously across the cervix and vaginal walls for one minute. CVL fluid was collected from the posterior fornixes and stored temporarily in a refrigerator (4°C) on ice until transfer to the laboratory. Protease inhibitor (Cocktail Set I, Calbiochem, Merck Millipore, Darmstadt, Germany) was added to one 1.5 ml aliquot for cytokine and chemokine testing and stored at −80°C until shipment, as previously described [107].

The urine aliquots and swab specimens were immediately stored at -80°C after arrival at the laboratory. All samples were transported frozen to the Netherlands for further testing at Leiden University Medical Center (LUMC). All laboratory analysis was performed blinded to any other participant data. Vaginal and cervical swab specimens were used for PCR detection of *Schistosoma* species; cervical swabs were used for detection of microbiota and

STIs by PCR; urine was used for CAA microscopic evaluation of the urine for *S. haematobium* eggs.

4.2.4 BILHIV study clinic-based portable colposcopy

Cervicovaginal images were captured with a portable colposcope (MobileODT, Tel Aviv, Israel) and were evaluated by an expert collaborator (EFK) for the presence of any of the four recognized FGS cervicovaginal manifestations: grainy sandy patches, homogenous yellow sandy patches, rubbery papules, and abnormal blood vessels [27]. Testing for STI was not performed at the point-of-care and participants with suspected STIs were offered syndromic management, as per local guidelines [108].

4.2.5 Urine microscopy

Urine aliquoting for quantification of CAA and urinalysis were performed on the day of specimen arrival at the laboratory. The remaining urine, up to 60mL per participant, was centrifuged in 15mL aliquots and examined by microscopy within 24 hours. The pellet from each 15mL urine aliquot (5 maximum) was evaluated for *S. haematobium* eggs. When a pellet contained at least one terminal-spined ovum, the participant was considered positive and the total number of counted eggs in the pellet was reported. Review of all positive and 10% of the negative specimens was conducted blinded by an expert for quality control. Dipsticks were used for analysis of haematuria and proteinuria (Multistix, Siemens, Germany).

4.2.6 Circulating anodic antigen

A lateral flow assay utilizing up-converting reporter particles for the quantification of CAA was performed on urine samples at LUMC [25, 31]. CAA is excreted from the gut of live schistosome worms into the host's bloodstream during active infection. CAA levels reflect the burden of live schistosomes and decline after successful treatment with praziquantel [49, 109]. Analysing the equivalent of 417 μL urine (wet reagent, UCAA***hT***417), a CAA value of >0.6 pg/mL was considered positive [109].

An up-converting reporter particle (UCP) lateral flow (LF) assay for the quantification of CAA in urine (0.4 mL) was performed at LUMC [31]. Urine CAA antigen levels are known

to reflect adult worm burden and decline after successful treatment with praziquantel [30, 49]. CAA comprises repetitive carbohydrate epitopes that efficiently bind the UCP reporter and the test-line on the LF strip via a CAA specific mouse monoclonal antibody. Interfering proteins were removed with a trichloroacetic acid (TCA) extraction by mixing 400 μL urine with 100 μL 12% (wt/vol) TCA. A centrifugation step removed the precipitate and 500 μL of the resulting clear TCA supernatant was concentrated to 20 μL using an 0.5 mL Amicon filtration device (Merck Inc.). Subsequently, the concentrate was mixed with a high salt lateral flow buffer containing the UCP reporter conjugated with monoclonal mouse anti-CAA antibody and incubated at 37°C for one hour. The same monoclonal mouse anti-CAA antibody is immobilized on a test line on a nitrocellulose membrane of an LF strip. These LF strips, comprised of a glass fibre sample pad, a nitrocellulose membrane, and an absorbent pad are inserted into microtiter plate wells with specimen for immunochromatography [31]. UCP technology uses a luminescent reporter particle that emits light upon excitation with 980 nm infrared light. Finally, LF strips are read with a modified Packard Fluorocount microtiter plate reader suited for IR excitation and LF strips. Measured signals are compared to a standard series with known amounts of CAA. For this assay, analysing the equivalent of 417 μL urine, a CAA value of 0.6 pg/mL was considered positive based on a series of negative controls (highest value plus 2 SDs).

4.2.7 Polymerase Chain Reaction for Schistosoma *spp.*

The internal transcribed spacer 2–based real-time PCR was performed for the detection of *Schistosoma* DNA in the clinical samples [110]. This PCR, using schistosome primers Ssp48F and Ssp124R and the double labelled probe Ssp78T, has been validated on its specificity [79, 90]. Appropriate positive and negative controls were included at each PCR run and, in addition, an internal control (Phocin herpes virus 1 (PhHV-1),10^3 plaque forming unit (PFU)/mL)) was added to each sample reaction for detection of potential inhibition of amplification [110]. For all specimens, DNA amplification and detection were performed with the CFX96 Real Time PCR Detection System (BioRad, California, USA). The output in threshold cycles (C_t) was analysed using BioRad CFX software.

Genital PCR was performed at the LUMC Department of Parasitology. After thawing, 1.5 mL of PBS was added to each tube containing a swab. After being vortexed for 10 seconds, the swabs were left at room temperature for another hour, after which the fluids were

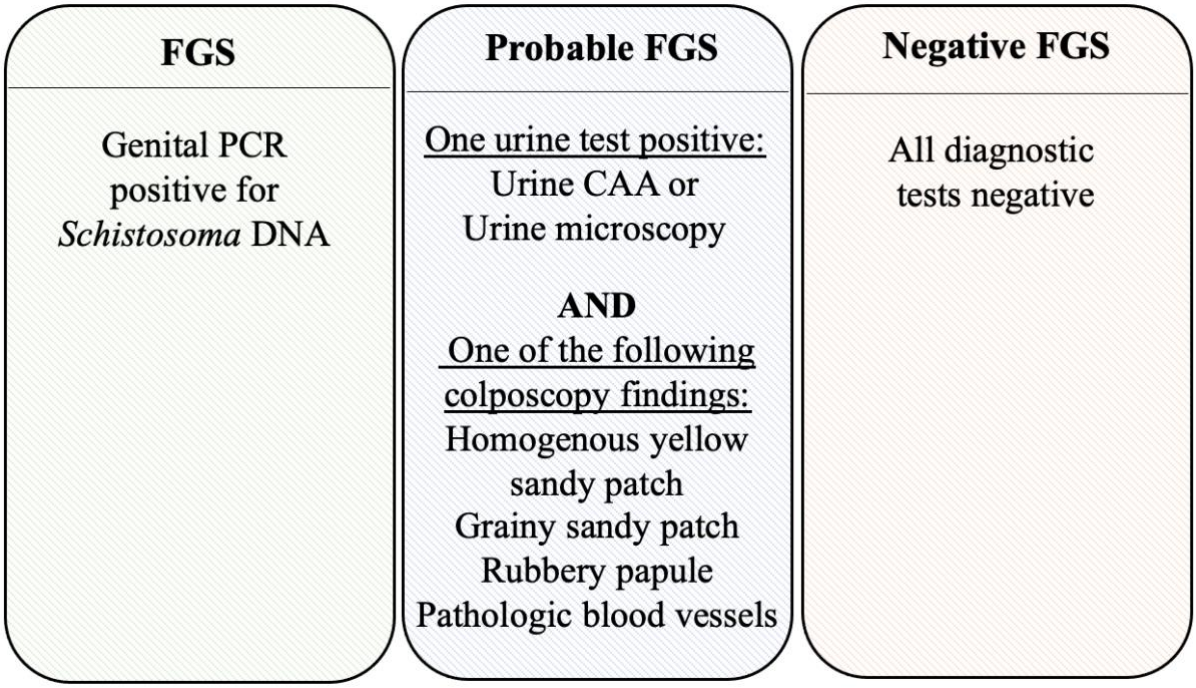
processed similar to the cervicovaginal lavage fluid. DNA extraction and PCR set up was performed at LUMC using a custom automated liquid handling station (Hamilton, Switzerland) [90]. DNA was extracted from 200µL of specimen using a proteinase K heating step followed by QIAamp spin columns (QIAGEN Benelux; Venlo, The Netherlands). Amplification consists of 15 min at 95°C followed by 50 cycles of 15 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C.

The output in C_t was analysed using BioRad CFX software. Negative and positive control samples were included in each amplification run. Parasite DNA load is categorized by the following pre-specified C_t threshold for all specimens processed by PCR (urine, cervical swab, vaginal swab, and CVL): any Ct-value observed was interpreted as positive and no Ct-value observed was interpreted as negative [111].

4.3 FGS definitions

For the purposes of this PhD thesis, we defined FGS using a variety of diagnostics to evaluate both schistosome infection (CAA and urine microscopy), and FGS (portable colposcopy and *Schistosoma* DNA on CVL and cervical and vaginal self-collected swabs) [25] . Participants were grouped by the outcomes of these diagnostic tests into three mutually exclusive categories (Figure 4.2). FGS was defined as at least one positive *Schistosoma* PCR on a genital specimen (cervical swab and/or vaginal swab and/or CVL). In participants who *Schistosoma* PCR negative on all genital specimens, *probable* FGS was defined as the presence of urinary schistosomiasis, detected with either urine CAA or urine microscopy, in combination with one of four clinical findings suggestive of FGS on any colposcope-obtained photograph [27]. *Negative* FGS was defined as negative results on all diagnostic assays. All participants with FGS (n=30) and all participants with *probable* FGS (n=25) were selected for characterization of the cervicovaginal microbiota and STIs by PCR on cervical swabs. Three *negative* FGS participants were selected for every FGS and *probable* FGS participant using a random number generator. The *negative* FGS participants were frequency matched by age to participants with FGS.

Figure 4.2 – Female genital schistosomiasis categories



adapted from Research Paper 4 (*Chapter 7*)

4.4 HIV-1

Laboratory-based fourth-generation HIV-1 testing (Abbott Architect HIV Ag/Ab Combo Assay) was performed for HPTN 071 (PopART) Population Cohort participants at each study visit [105]. Additional testing using antigen/antibody screening tests, a discriminatory test, and an HIV-1 RNA test was used to confirm incident HIV-1 infection, as previously described [112].

4.5 Cervicovaginal microbiota characterization and STI detection

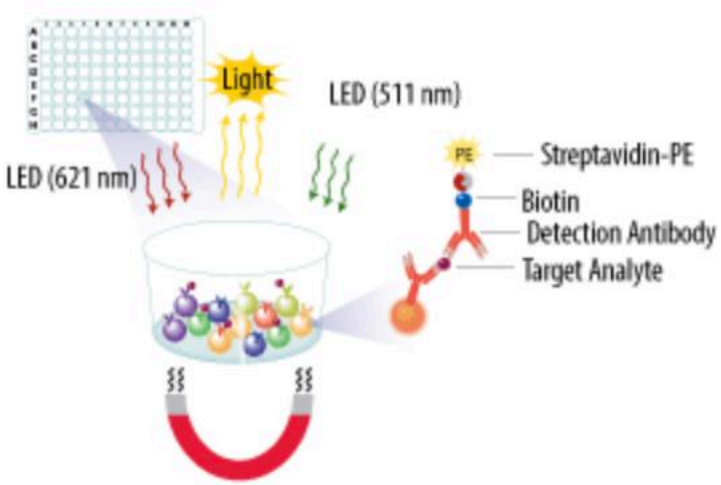
We quantified key markers vaginal health (*L. crispatus*), BV (*G. vaginalis* and *A. vaginae*), as well as *L. iners* (a highly prevalent lactobacillus with an enigmatic role), *C. albicans* and STIs (*Chlamydia (C.) trachomatis*, *Neisseria (N.) gonorrhoeae*, *Mycoplasma (M.) genitalium*, and *Trichomonas (T.) vaginalis).* STIs were quantified by quantitative PCR using the S-DiaCTNG™ (for *C. trachomatis* and *N. gonorrhoeae*) and S-DiaMGTV™ kits (for *M. genitalium* and *T. vaginalis*) (both Diagenode Diagnostics, Seraing, Belgium) on DNA obtained from cervical swabs at Ghent University (Ghent, Belgium) according to the manufacturer's instructions. Quantification of *Atopobium vaginalis*, *Gardnerella vaginalis*, *Lactobacillus crispatus*, *L. iners* and *Candida* species was performed in the Laboratory

Bacteriology Research, Ghent using the LightCycler480® and the LightCyclerR 480 Software Version 1.5 (Roche, Basel, Switzerland). The specific species of *Candida* were determined based on melting peaks. Amplification was carried out on the LightCycler480® and the LightCyclerR 480 Software Version 1.5 (Roche, Basel, Switzerland). To quantify each of the target species, standard curves were constructed from a tenfold dilution series of DNA from reference strains. The genomic concentrations were calculated using the described genomic sizes of the type strains. The concentration of each species was expressed as genome equivalents per mL (ge/mL) [113].

4.6 The MAGPIX© bioanalyzer

Luminex MAGPIX© technology uses magnetic color-coded beads and an antibody-capture system to quantify the amount of unknown analyte in a clinical specimen [114]. Within a test kit, specific antibodies are coupled to magnetic microbeads. When a clinical specimen is added, the microbead-bound antibodies bind with the analyte of interest. Similar to a sandwich-ELISA, specific biotin-bound antibodies then bind the clinical specimen's epitope of interest that has been captured on the magnetic bead [115, 116]. Streptavadin-phycoerythrin (a fluorochrome) has a high affinity for biotin and is used to detect biotinylated antibody binding. Often this reaction occurs in a 96-well plate [114], which is then placed directly within a Luminex MAGPIX© bioanalyzer. The beads are carried into a chamber where a magnet captures the beads in a monolayer and uses light emitting diode (LED) technology to identify the beads and the amount of fluorescence present [117]. A red LED light excites dyes that allow microbead identification. A green LED excites the streptavadin-phycoerythrin and fluorescence is captured with a charge-coupled device (CCD) camera, this is the mean fluorescence intensity (MFI) (Figure 4.3) [117].

Figure 4.3 – The use of LED technology to identify and excite magnetic beads in the MAGPIX bioanalyzer



Adapted from [117]

4.6.1 Multiplex bead-based assays

Prior to beginning work in the laboratory, I obtained permission from Occupational Health to work with specimens known to contain *S. haematobium* DNA (Appendix 3) and performed a risk assessment, which was certified by my project supervisor and the Faculty Safety Supervisor (Appendix 4). Luminex MAGPIX© was used to measure concentrations of seventeen soluble cytokines and chemokines using MILLIPLEX Human Cytokine/Chemokine Magnetic Bead kits (Merck Millipore, Darmstadt, Germany) according to the manufacturer's instructions and recommendations for dilute samples, i.e. CVL. The concentrations of eotaxin (CCL-11), interferon-gamma (IFN-γ), interleukin (IL)-10, IL-13, IL-15, IL-17A, IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8 (CXCL-8), IP-10 (CXCL-10), monocyte chemoattractant protein (MCP-1) (CCL-2), macrophage inflammatory protein-1 (MIP-1α) (CCL-3), macrophage inflammatory protein-1β (MIP-1β) (CCL-4) and tumour necrosis factor-α (TNF-α) were measured in undiluted CVL in duplicate. I set the HCYTOMAG-60K kit up on a 96-well plate employing four different types of wells: standards, quality control (QC), background, and unknowns. The reagent for the standard is provided in the test kit and is prepared in serial dilutions. The standard contains a known concentration of the analytes of interest and is used to calculate standard curves and convert specimen and QC MFI into concentrations. The quality control wells contain specific concentrations of the analytes of interest and aid in minimizing between-plate variations in cytokine and chemokine

concentrations. The background wells contain only assay buffer and are used to subtract any non-specific fluorescence. The CVL specimens collected in the BILHIV study were the unknowns. Additionally, equal proportions of specimens with FGS, *probable* FGS, and *negative* FGS were distributed across six 96-well plates [118].

Briefly, we wetted a 96-well microtitre plate by adding wash buffer to each well of the plate [116]. The plate was sealed and mixed on a plate shaker for 10 minutes at room temperature. The wash buffer was then decanted and each standard and control was added to the appropriate wells followed by assay buffer. Neat CVL and assay buffer was added to sample and background wells, respectively. Assay buffer was added into the background wells. The mixed beads were vortexed and added to each well. The plates were sealed, wrapped in foil, and incubated with agitation on a plate shaker overnight at 4ºC [116]. Maintaining the plate on a magnet to retain microbeads and bound specimen, the contents of the well were removed, and the plates were washed two times with wash buffer. We then added detection antibodies into each well and sealed, covered with foil and incubated with agitation on a plate shaker for 1 hour at room temperature. We then added streptavidin-phycoerythrin to each well containing detection antibodies [116]. We then performed 2 washes and added drive fluid to all wells and resuspended the beads on the plate shaker for 5 minutes. We then ran the plates on the MAGPIX© bioanalyzer with a 100µL acquisition volume [116].

With xPONENT software (version 4.2), the MFI was measured, background-adjusted, and converted into analyte concentrations using a 5-parameter logistic regression (5PL) equation to interpolate standard curves. The lower limit of detection was between 0.26 and 5.66 pg/ml for the 17 cytokines and chemokines measured. Cytokine or chemokine concentrations below the lower limit of quantification (LLOQ) were imputed to be the midpoint of the lowest concentration for each analyte and zero, and concentrations above the upper limit of quantification were imputed as the highest concentration for each analyte.

4.6.2 Quality control

I identified the total bead count in each well and each well had no less than 50 beads. I created histograms for each analyte and also inspected standard curves visually for each analyte across plates for the presence of outliers. The xPONENT software (version 4.2) used a 5PL equation to determine concentrations of analytes from the standard curve. I generated my own 5PL and 4 parameter logistic (4PL) regression equations and standard curves. The data generated from the 5PL equation best described the data. Additionally, my 5PL equation, and the subsequent standard curves I generated were similar to those generated by xPONENT (r^2>0.9). Thus, ultimately, I used concentrations calculated by xPONENT for the remainder of the analysis.

Levey-Jennings plots can be used to show changes in quality-control metrics across plates [119]. I used these graphs to display show the mean MFI, plus/minus one and two standard deviations, per analyte and each standard at 16 pg/mL, 80pg/mL and 400pg/mL on the standard curve. There were no observed outliers. The coefficient of variation (CV) can also be used to identify changes across duplicate wells and across plates. *A priori* I specified a mean CV of <15% for plates to indicate an acceptable level of heterogeneity, and no mean plate CVs exceeded this threshold. I also evaluated duplicate wells and no duplicate wells exceeded a CV of 25%. I also included two control reagents across the six plates, as recommended by the manufacturer.

Vaginal cytokines and chemokine levels may be low when using cervicovaginal lavage samples. Thus, I validated assay results by confirming an association of the pro-inflammatory cytokines IL-1α, IL-1β, and TNF-α with the presence and concentrations of *Gardnerella vaginalis* and *Atopobium vaginae*, bacteria associated with bacterial vaginosis. I used the Wilcoxon rank-sum test to compare the concentrations of the pro-inflammatory cytokines by presence and absence in participants with *G. vaginalis* and by presence and absence with *A. vaginae.* Additionally, the pro-inflammatory cytokine concentrations were also compared by *G. vaginalis* and *A. vaginae* concentration levels using the Kruskal-Wallis test. Results were similar by both analysis strategies. The results of pro-inflammatory cytokine concentrations by presence and absence of *G. vaginalis* and *A. vaginae* are presented in Chapter 6 (Supplementary Figures 8 and 9, respectively).

4.7 The role of the Open Data Kit in data collection

The BILHIV parent study operated in participant homes, the cervical cancer clinic, and the clinical laboratory. Data were collected across these three locations using xforms-based questionnaires on handheld tablets. Community workers conducted questionnaires at home visits and entered the participant's data directly into handheld tablets. The midwives used a tablet-based clinical research form to capture information regarding the participant's clinical encounter. The laboratory technician also used a handheld tablet device to enter specimen and processing-related data. I used the ODK, an open-source software to collect and manage data [120]. As there was not reliable internet connectivity in Livingstone, ODK allowed us to collect data offline and then sync data to the LSHTM server when internet connectivity was available. All data were stored in the LSHTM secure server.

4.8 Statistical analysis plan

Full details of the statistical analyses are included in each individual research paper, and this section contains an overview of the statistical methods applied in this PhD thesis. For all analyses, participant characteristics were summarized by median and interquartile range (IQR) for continuous variables, and by frequency and percentage for categorical variables. Differences in characteristics between the FGS categories were evaluated using Fisher's exact or chi-squared tests. In each manuscript, the primary comparison was between FGS and *negative* FGS women. In Research Paper 2 (*Chapter 5* – FGS, the cervicovaginal microbiota, and STI) and Research Paper 3 (*Chapter 6* – FGS and cervicovaginal immune activation) a secondary analysis compared FGS and probable FGS groups with the *negative* FGS participants. In Research Paper 4 (*Chapter 7* – FGS and HIV-1 incidence), to be able to use all HIV-1 seroconversion data, we created a "probable/possible FGS" category, defined as the presence of either a positive urine diagnostic (CAA or microscopy) or one of four cervicovaginal manifestations suggestive of FGS on portable colposcopy, or both with a negative genital PCR. With FGS, probable/possible FGS, and *negative* FGS as the exposure variables, I calculated the relative risk of HIV-1 seroconversion per unit of the exposure variable as a continuous variable. For each of the Research Papers, I performed two exploratory analyses to evaluate the association of schistosome infection intensity with study outcomes. The first exploratory analysis compared participants with "PCR-defined FGS" and a moderate/high *Schistosoma* DNA concentration (Ct <35) with those in the *negative* FGS

group. The second exploratory analysis compared participants with "PCR-defined FGS" with ≥2 positive genital PCR specimens with those in the *negative* FGS group.

4.8.1 Research paper 2 (Chapter 5) – FGS, the cervicovaginal microbiota and STIs

Log concentration means, medians, and prevalence were calculated by t-tests, ranksum test, chi-squared test, respectively, for each reported species when at least 20% of participants had results detectable by PCR (>20% prevalence). For species with <20% prevalence, p-values comparing presence/absence between FGS groups was calculated with the Fisher's exact test. We evaluated for potential confounding using linear and logistic regression. Species with >20% prevalence were log-transformed and evaluated by linear regression in univariate and multivariate analysis and were adjusted for age, community of residence and education. We calculated the crude and adjusted odds of presence/absence of each species by FGS group with logistic regression and adjusted for age.

4.8.2 Research Paper 3 (Chapter 6) – FGS and cervicovaginal immune activation

Characteristics of participants were summarized and compared between "PCR-defined FGS" and *negative* FGS groups. In the initial analysis, cytokines were considered individually. For cytokines or chemokines with at least 70% of sample results above the LLOQ, differences in median cytokine or chemokine concentrations were compared between FGS categories with the Wilcoxon-Mann-Whitney test. For cytokines with <70% of the samples above the lower limit of quantification (IL-5, IL-13, IL-15, and TNF-a), these cytokines were evaluated as presence/absence and were compared between FGS categories by the chi-squared test. Next, cytokine and chemokine responses were compared between FGS and *negative* FGS participants. Finally, FGS and *negative* FGS participants were compared using multivariable regression, with adjustment for potential confounders. Cytokines with <70% of the samples above the LLOQ were evaluated by logistic regression and were adjusted for age [107].

I then investigated combining information from multiple cytokines. Initially, I used Spearman's rank correlation coefficient for each analyte pair to understand patterns of association between cytokines. I then used dimension reduction techniques to evaluate the possibility that groups of cytokines may be correlated and/or associated with FGS in a similar way. I used principal components analysis to linearly transform the original set of correlated MAGPIX-generated analytes to a new set of variable, "components", which are uncorrelated,

with the first principal component capturing the largest amount of variation from the original variables [121]. The means of the first principal components were taken forward for additional comparisons between FGS groups.

4.8.3 Research paper 4 (Chapter 7) – FGS and HIV-1 incidence

Women who were HIV-1 positive at HPTN 071 (PopART) baseline were excluded from the analysis of this objective. HIV-1 incidence was calculated as the number of seroconversions per 1,000 person-years of follow up. Participants contributed person-time for the calculation of HIV-1 incidence starting with their first HIV-1 test and ending at date of HIV-1 seroconversion for those who seroconverted, or at the date of last follow-up or the end of scheduled follow-up (whichever occurred earliest) for women who did not seroconvert. HIV-1 seroconversion was assumed to occur at the midpoint between the last negative and the first positive HIV-1 test. Associations of risk factors with incident HIV-1 infection were calculated as rate ratios and 95% confidence intervals, estimated using exact Poisson regression in univariable and multivariable analysis.

4.9 Ethical considerations

The parent study and the sub-studies were approved by the University of Zambia Biomedical Research Ethics Committee (reference 011-08-17, Appendix 5 and Appendix 6), the Zambia National Health Research Authority and the London School of Hygiene and Tropical Medicine Ethics Committee (reference 14506, Appendix 7 and Appendix 8). Permission to conduct the study was given by Livingstone District Health Office and the superintendent of Livingstone Central Hospital.

Chapter 5

Research paper 2

Chapter 5 – Associations of female genital schistosomiasis with the cervicovaginal microbiota and sexually transmitted infections in Zambian women.

Citation: Sturt AS, Webb EL, Himschoot L, Phiri C, et al. Associations of female genital schistosomiasis with the cervicovaginal microbiota and reproductive tract infections in Zambian women. Open Forum Infectious Diseases – Submitted and currently undergoing peer review.

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SECTION A – Student Details

Student ID Number	1702513	**Title**	Dr
First Name(s)	Amy		
Surname/Family Name	Sturt		
Thesis Title	Association of Female Genital Schistosomiasis with the Cervicovaginal Microbiota and Sexually Transmitted Infections in Zambian Women		
Primary Supervisor	Amaya Bustinduy		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Open Forum Infectious Diseases
Please list the paper's authors in the intended authorship order:	Amy S. Sturt; Emily L. Webb; Lisa Himschoot; Comfort R. Phiri; Joyce Mapani; Maina Mudenda; Eyrun F. Kjetland; Tobias Mweene; Bruno Levecke; Govert J. van Dam; Paul L. A. M. Corstjens; Helen Ayles; Richard J. Hayes; Lisette

	van Lieshout; Isaiah Hansingo; Suzanna C. Francis; Piet Cools; Amaya L. Bustinduy
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	

SECTION E

Student Signature	Amy Sturt
Date	April 14, 2021

Supervisor Signature	Amaya Bustinduy
Date	April 30, 2021

Association of Female Genital Schistosomiasis with the Cervicovaginal Microbiota and Sexually Transmitted Infections in Zambian Women

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Summary:

The association of female genital schistosomiasis with the cervicovaginal microbiota and sexually transmitted infections is not well-described. We report weak evidence of an association between *Trichomonas vaginalis* and FGS, with a stronger association in women with higher burden FGS infection.

Keywords: Female genital schistosomiasis, *Schistosoma haematobium*, cervicovaginal microbiota, sexually transmitted infection

Manuscript Word Count: 3014

Abstract word count: 198

Number of Figures: 1

Number of Tables: 4

Abstract

Background

The cervicovaginal microbiota, including sexually transmitted infections (STI), have not been well-described in female genital schistosomiasis (FGS).

Methods

Women (aged 18-31, sexually active, non-pregnant) were invited to participate at the final follow-up of HPTN 071 (PopART) Population Cohort in January-August 2018. We measured key species of the cervicovaginal microbiota (*Lactobacillus crispatus*, *L. iners*, *Gardnerella vaginalis*, *Atopobium vaginae* and *Candida*) and STI (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* and *Mycoplasma genitalium*) using quantitative PCR (qPCR). We evaluated associations of microbiota and STI presence and concentration with FGS (qPCR-detected *Schistosoma* DNA in any of three genital specimens).

Results

The presence and concentration of key cervicovaginal species did not differ between participants with (n=30) or without FGS (n=158). A higher proportion of participants with FGS had *T. vaginalis* compared to FGS negative women (p=0.08), with further analysis showing that *T. vaginalis* was more prevalent among women with ≥2 *Schistosoma* qPCR positive genital specimens (50.0%, 8/16) than among FGS negative women (21.5% 34/158, p=0.01).

Conclusions

We found weak evidence of an association between *T. vaginalis* presence and FGS, with a stronger association in women with a higher burden FGS infection. Additional research is needed on potential between-parasite interactions, especially regarding HIV-1 vulnerability.

Introduction

Female genital schistosomiasis (FGS), caused when eggs from the waterborne parasite *S. haematobium* are entrapped in genital tissue, is prevalent in sub-Saharan Africa and is associated with adverse reproductive outcomes, including ectopic pregnancy, infertility, and prevalent HIV-1 [1, 2]. The cervicovaginal environment has been described as "optimal" when it is dominated by lactic-acid producing lactobacilli, commensal microorganisms that adhere to an intact vaginal squamous epithelium [3, 4], protecting against pathogens by acidifying the vagina and producing antimicrobial substances such as bacteriocins [3]. Bacterial vaginosis (BV) and vulvovaginal candidiasis are examples of "non-optimal" microbiota, characterized by a shift from lactobacilli dominance to an increase in anaerobic species or yeast [4]. BV is prevalent in women in sub-Saharan Africa [5, 6] and has important sexual and reproductive health consequences, including increased risk of pelvic inflammatory disease, and adverse pregnancy outcomes such as pre-term delivery [5, 7], a leading cause of under-five mortality in sub-Saharan Africa [8]. BV is also strongly associated with HIV-1 acquisition and other sexually transmitted pathogens infecting the genital tract, [5, 9, 10] yet the relationship between cervicovaginal microbiota and FGS is not well characterized.

Studies employing 16S rRNA sequencing to evaluate the cervicovaginal microbiota suggest that high-intensity urinary *S. haematobium* infection, in the absence of investigation for genital involvement, may alter cervicovaginal microbiota diversity [11]. In addition, studies reporting qPCR-defined FGS have either not investigated or reported STI prevalence [12-14] or STI prevalence has been correlated with visual FGS findings [15]. In this cross-sectional study we utilized qPCR to detect *Schistosoma* DNA in the female genital tract and evaluated the association

of PCR-defined FGS with the concentration and presence of key markers of the cervicovaginal microbiota, including STI.

Methods

Study setting and participants

The cross-sectional bilharzia and HIV (BILHIV) study [16] was nested in HPTN 071 (PopART), a cluster randomized trial to measure the impact of an HIV-1 combination prevention package [17]. In HPTN 071 (PopART), HIV-1 incidence was measured in a Population Cohort at baseline, 12, 24, and 36 months [17]. Between January and August 2018, after the 36-month HPTN 071 (PopART) visit, community workers made home visits to women expressing interest in the BILHIV study [16]. Eligible women were 18-31 years, not pregnant, sexually active, and resident in one of two urban communities that participated in HPTN 071 (PopART) in Livingstone, Zambia.

Home and clinic-based sample collection

The home visit included written informed consent, a questionnaire, genital self-sampling (cervical and vaginal), and urine specimen collection, as previously described [16]. Enrolled women not currently menstruating were invited to attend Livingstone Central Hospital cervical cancer clinic, where midwives performed cervicovaginal lavage (CVL). After speculum insertion, a bulb syringe was used to flush normal saline (10 mL) across the cervix and vaginal walls for one minute. CVL fluid was collected from the posterior fornices (S1-Text). CVL, vaginal and cervical swab specimens were used for quantitative PCR (qPCR) detection of *Schistosoma*; cervical swabs were

used for characterization of the microbiota and STI by qPCR; urine was used for detection of circulating anodic antigen (CAA) and *S. haematobium* eggs by microscopy.

Cervicovaginal images were captured with a portable colposcope (MobileODT, Tel Aviv, Israel) and were evaluated (EFK) for any of the four recognized FGS cervicovaginal manifestations: grainy sandy patches, homogenous yellow sandy patches, rubbery papules, and abnormal blood vessels [18]. Women with at least one of these manifestations [18] or with any positive urine or genital *Schistosoma* diagnostic were treated free of charge with 40 mg/kg praziquantel. Testing for STI was not performed at the point-of-care and participants with suspected STI were offered syndromic management, as per local guidelines [19].

HIV-1

Laboratory-based fourth-generation HIV-1 testing (Abbott Architect HIV Ag/Ab Combo Assay) was performed for HPTN 071 (PopART) Population Cohort participants at each study visit [17].

Urine microscopy and Circulating Anodic Antigen

Urine was centrifuged and examined by microscopy for *S. haematobium* eggs. The participant was considered positive if a pellet contained at least one *S. haematobium* egg [16]. A lateral flow assay utilizing up-converting reporter particles for the quantification of CAA was performed on urine samples, as previously described [16, 20]. Analyzing the equivalent of 417 μL urine (wet reagent, UCAA***hT***417), a test result indicating a CAA value >0.6 pg/mL was considered positive [21].

qPCR for detection of Schistosoma DNA

Detection of the *Schistosoma*-specific internal-transcribed-spacer-2 (ITS2) target by qPCR was performed at LUMC, as previously described (S1-Text) [16, 22]. DNA extraction of 200 µL of CVL, cervical or vaginal swab fluid was done with QIAamp spin columns (QIAGEN Benelux; Venlo, The Netherlands) according to manufacturer's guidelines. The qPCR output was reported in cycle threshold values (Ct-values) and parasite DNA loads were categorized by the following pre-specified values: high (Ct<30), moderate (30≤Ct <35), low (35≤Ct<50) and negative (no amplification) [23].

Cervicovaginal microbiota characterization and STI detection

We quantified *Lactobacillus crispatus* as a key marker of vaginal health. Additionally, we characterized BV (*Gardnerella vaginalis* and *Atopobium vaginae*), as well as *L. iners* (an enigmatic and highly prevalent lactobacillus), *Candida* spp. and STI (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis)*. STI were quantified by qPCR using the S-DiaCTNG™ (for *C. trachomatis* and *N. gonorrhea*) and S-DiaMGTV™ kits (for *M. genitalium* and *T. vaginalis*) (Diagenode Diagnostics, Seraing, Belgium) on DNA from cervical swabs at Ghent University (Ghent, Belgium) according to the manufacturer's instructions. Quantification of *A. vaginae*, *G. vaginalis*, *L. crispatus*, *L. iners* and *Candida* species was performed in the Laboratory Bacteriology Research, Ghent using the LightCycler480® and Software Version 1.5 (Roche, Basel, Switzerland) (S1-Text). The concentration of each species was expressed as genome-equivalents per mL (ge/mL) [24].

FGS Definitions

In this study we employed various diagnostic tests to evaluate urinary schistosome infection (CAA and urine microscopy), and FGS (portable colposcopy and *Schistosoma* DNA on CVL and genital swabs). As previously described [25], participants were grouped by diagnostic test results into three mutually exclusive categories: FGS, at least one positive *Schistosoma* qPCR on a genital specimen (cervical swab, vaginal swab and/or CVL); FGS *negative*, negative results on all diagnostic methods; *probable* FGS, genital *Schistosoma* qPCRs negative but urinary schistosomiasis positive (as defined above), in combination with one of four clinical findings suggestive of FGS on any colposcope-obtained photograph [18].

Patient consent statement

The study was approved by the University of Zambia Biomedical Research Ethics Committee (011-08-17), the Zambia National Health Research Authority and the London School of Hygiene and Tropical Medicine Ethics Committee (14506). Permission to conduct the study was given by Livingstone District Health Office and the Livingstone Central Hospital superintendent. Participants provided written informed consent.

Statistical Methods

All participants with FGS (n=30) and all participants with *probable* FGS (n=25) were selected for characterization of the cervicovaginal microbiota and STI by qPCR on cervical swabs. Three FGS *negative* participants were selected for every FGS and *probable* FGS participant using a random number generator. The FGS *negative* participants were frequency matched by age to participants with FGS (age groups 18-19, 20-21, 22-23, 24-25, 26-27, 28-29, 30-31).

Participant characteristics were summarized by median and interquartile range (IQR) for continuous variables, and by frequency and percentage for categorical variables. Differences in characteristics between FGS categories were evaluated using Fisher's exact or chi-squared tests. For cervicovaginal microbiota and STI species with at least 20% of sample results detectable by qPCR (i.e. ≥20% prevalence), p-values for comparison of presence, median (IQR), and $\log_{10}$ concentration mean between FGS and FGS *negative* groups were calculated from chi-squared test, ranksum test, and t-test, respectively. For species with <20% prevalence, species presence was compared between FGS groups using Fisher's exact test. To enable investigation of potential confounding, concentrations of cervicovaginal microbiota with ≥20% prevalence were log-transformed to normalize their distribution, and linear regression used to evaluate the association between FGS and mean log concentration of each organism (ge/mL) in univariable and multivariable analysis. We developed a causal framework (Figure 5.S1) to inform our minimal adjustment set, and adjusted for age, education and community of residence. For all species, logistic regression was used to calculate crude and adjusted odds ratio (OR) for presence versus absence by FGS group; due to the relatively low number of participants with detectable concentrations, logistic regression analyses only adjusted for age. Given the exploratory nature of this work, we did not correct for multiple comparisons.

Our primary analysis focused on the detection of *Schistosoma* DNA in the genital tract (FGS versus FGS *negative*). A secondary analysis compared FGS and *probable* FGS groups with the FGS *negative* participants. To evaluate the possible association between FGS burden and changes in presence and concentration of cervicovaginal key species, two ad-hoc analyses were performed: (1) participants with ≥2 genital samples with detectable *Schistosoma* DNA were compared with the FGS *negative* group, (2) participants with a moderate/high genital *Schistosoma* DNA

concentration (Ct <35 in at least one of three samples) were compared with the FGS *negative* group. Data were analyzed using STATA 15.1 (Stata Corporation, College Station, TX).

Results

A total of 603 eligible women were enrolled and 213 (35.3%) were included in this study (Figure 5.S2). Of those included, 14.1% (30/213) had FGS, defined by a positive genital *Schistosoma* qPCR from any of the following sites: 9.4% (20/213) cervical swab, 7.0% (15/213) vaginal swab, and 6.6% (14/211) CVL. Of participants with FGS, 53.3% (16/30) had *Schistosoma* qPCR detected from multiple sites and 53.3% (16/30) had moderate/high genital Schistosoma DNA loads, these groups overlapped by 12 participants. Twenty-five women had *probable* FGS, and 74.2% (158/262) of the women who were negative on all diagnostic tests were randomly selected for inclusion in this study.

Baseline characteristics

The majority of the participants were married/cohabitating, had received some secondary education, and were using hormonal contraception (Table 5.1). At the conclusion of HPTN 071 (PopART), HIV-1 prevalence was 17.4% (37/213) among the women included in this study and one-third had at least one STI (Table 5.1). Urinary schistosome infection, defined as either a positive urine microscopy (11.7%, 25/213) and/or positive CAA (20.7%, 44/213), was reported in 21.6% (46/213) of participants. There were differences between the three categories of FGS status for age (p=0.002), marital status (p=0.04), education (p=0.06), and employment (p=0.04) with participants in the *probable* FGS group more likely to be older, employed, and married than FGS and FGS *negative* participants. There was strong evidence of a difference in community of residence by FGS status (p<0.001) with participants with FGS and *probable* FGS more likely to

live in Community A than participants in the FGS *negative* group (Table 5.1). Other characteristics were similar by FGS status.

Primary comparison: FGS vs FGS negative

Concentrations of evaluated species are shown in Figure 5.1. Compared to FGS *negative* women, there was no evidence of a difference in the presence or concentration of cervicovaginal *L. crispatus*, *L. iners*, *A. vaginae*, *G. vaginalis* or *Candida* spp. in participants with FGS (Table 5.2). There was weak evidence that a higher proportion of participants with FGS had *T. vaginalis* present (OR 2.11, 95% CI: 0.92 – 4.86, p=0.08, Table 5.S2). This association remained after adjusting for age (Table 5.S1). Otherwise, compared to FGS *negative* women, the presence and concentration of other STI was similar in women with FGS (Table 5.2).

Combining FGS and *probable* FGS groups, participants with FGS/*probable* FGS similarly had a higher prevalence of *T. vaginalis* compared to FGS *negative* participants (Table 5.S2, p=0.05). Otherwise, compared to FGS *negative* women, the presence and concentrations of cervicovaginal microbiota and the presence of STI was similar in women with FGS/*probable* FGS compared to FGS *negative* participants (Table 5.S2).

Ad-hoc Analysis* – Schistosoma *DNA concentration

In participants (n=16) with FGS and moderate/high genital *Schistosoma* DNA concentration (*Schistosoma* qPCR Ct<35) the presence of *T. vaginalis* was higher than among the FGS *negative* participants (p=0.01, Table 5.3). Women with FGS and a moderate/high *Schistosoma* DNA

concentration (Ct<35) had a higher mean concentration of *G. vaginalis* compared to FGS *negative* women (p=0.03, Table 5.3).

Ad-hoc analysis – clinical disease burden

In an ad-hoc analysis, participants (n=16) with a higher FGS burden, defined as ≥2 *Schistosoma* qPCR positive genital specimens, had higher prevalence of *T. vaginalis* compared to FGS *negative* participants (p=0.01, Table 5.4). There was also evidence of a difference in the median concentration and the mean log-concentration of *L. iners* (both p=0.03) compared to FGS *negative* women, with lower levels among the higher FGS burden group (Table 5.4).

Discussion

In this study, we describe the association of FGS with the cervicovaginal microbiota, including lactobacilli, *Candida* spp., markers of a "non-optimal" cervicovaginal environment, and STI. We did not find evidence that the presence or concentration of key cervicovaginal species was associated with FGS. While FGS was not associated with *C. trachomatis*, *M. genitalium*, or *N. gonorrhoeae*, there was weak evidence of an association of *T. vaginalis* presence with FGS, which remained after adjusting for age. This association was also present when participants with FGS and *probable* FGS were combined and is strengthened in the ad-hoc analyses of participants with higher burden FGS.

We performed two ad-hoc analyses. High-intensity *S. haematobium* infection, in the absence of evaluation for FGS, has been associated with higher cervicovaginal alpha diversity [11]. Thus, first we investigated whether *Schistosoma* DNA concentrations might be associated with the

cervicovaginal microbiota in 16 participants with a higher FGS burden, indicated by moderate/high genital *Schistosoma* DNA concentrations. In this ad-hoc analysis, we found that the *G. vaginalis* log-concentration mean was higher in women with a higher FGS burden. Participants underwent CVL when they were not menstruating, and we have previously described that 66.2% (139/210) of women in this cohort had detectable CVL hemoglobin. Iron sources, like hemoglobin, are often required for bacterial growth [26]. *G. vaginalis* is well adapted to harvest iron from the environment [27] and higher concentrations of *G. vaginalis* coincide with menses [26]. Cervical tissue in women with FGS is more vascularized than non-egg containing tissue and thus, the abnormal cervical vessels and contact bleeding seen in clinical FGS provides a plausible link to increased concentrations of *G. vaginalis* in high-burden FGS [28].

In a second ad-hoc analysis, we examined participants with multiple qPCR-positive genital specimens as a potential proxy marker of high-burden FGS and found that reduced *L. iners* concentration (median and log mean) was associated with high-burden FGS. The cervicovaginal microbiota was characterized with 16S rRNA sequencing in Tanzanian women with *S. haematobium* infection (n=16). Although power was limited, women with high-intensity *S. haematobium* infection had reduced abundance of *L. iners* compared to women with low-intensity infection, albeit without evidence of a difference (p=0.39) [11]. Though a Lactobacillus species, *L. iners* can be present in both 'optimal' and 'non-optimal' cervicovaginal microbiomes [29]. *L. iners* does not produce D-lactic acid, which may influence cervical mucus thickness and permeability [29, 30]. Additionally, *L. iners'* small genome size may suggest it has adapted well to relying on the host environment [29]. Further research is needed to evaluate the relationship between *L. iners* presence and concentration in FGS.

In both ad-hoc analyses, we found that *T. vaginalis* presence was higher among the participants with a higher FGS burden. Our finding supports results from a small South African study (n=45) that reported an association between FGS (diagnosed by identification of sandy patches on colposcopy) and *T. vaginalis* presence in young women (ages 15-23) [31]. Acquisition of *T. vaginalis* and *S. haematobium* may share common risk factors, like age and socioeconomic status [16]. We have previously described higher FGS prevalence amongst younger age groups [16]. Epidemiologic data from Zambian adolescents, sex-workers and pregnant women (aged 13-45 years) describes a *T. vaginalis* prevalence between 24.6-33.2% [32], consistent with the prevalence in our population (24.9%). While *S. haematobium* is geographically restricted to Africa and the Middle East, it is associated with poverty [33] and is acquired through contact with cercariae infested water [2]. *T. vaginalis* is primarily sexually transmitted, is prevalent worldwide, with the highest prevalence in women from low-income countries [34]. Macrophage polarization can be influenced by the local immune environment, schistosomes, and *T. vaginalis* [35, 36]. We have previously shown that, compared to FGS *negative* women, high-burden FGS is associated with higher concentrations of cervicovaginal Th2 cytokines [25]. A mouse model of urogenital *S. haematobium* infection suggested that the Th2 immune environment may be associated with delayed pathogen clearance [37]. Thus, further research is needed regarding the interaction between the immune environment and macrophage phenotypes in FGS and their role in potentially influencing *T. vaginalis* persistence.

Our study is the first to evaluate cervicovaginal microbiota and STI in FGS defined by qPCR. This is particularly relevant in a population of sexually active, non-pregnant women in the context of

high HIV-1 prevalence. Many studies evaluating the cervicovaginal microbiota apply either a PCR-based or a 16s rRNA sequencing approach. A strength of the qPCR-based approach was the ability to determine concentrations of the species of interest. Additionally, since 16s rRNA only identifies bacterial pathogens, the use of a PCR-based technique allowed us to identify a protozoan (*T. vaginalis*) and yeast (*Candida* spp.) that would not be detected by 16s rRNA sequencing. A limitation of the PCR-based approach is that we were only able to identify the organisms for which we had chosen primers, whereas 16s rRNA sequencing allows a broader analysis of cervicovaginal microbial populations [38]. A limitation of the 16s rRNA sequencing approach is that 16s rRNA generates relative rather than absolute bacterial abundance [39]. Other experimental limitations of a 16s rRNA approach include bias that can potentially be introduced due to primer affinity and guanine/cytosine composition, with the outcome that not all 16s genes amplify similarly [39]. Additionally, computational limitations of 16s rRNA sequencing include challenges with quality control, genome assembly, and taxonomic classification [38].

The study was conducted in an urban location with relatively low *S. haematobium* prevalence, thus the numbers of FGS cases in the primary and ad-hoc analyses were small, limiting precision in the effect sizes. This analysis also included multiple statistical comparisons; thus, we focused on the species that showed a consistent pattern of association across primary and ad-hoc analyses, rather than over-interpreting significance testing for any one species in insolation. Evidence for these associations in the ad-hoc analyses should be viewed as hypothesis generating. Additionally, the cross-sectional study design limited our ability to assess FGS duration or the long-term impact on the prevalence and concentrations of key species or STI. There were a number of behavioral and biological factors that were not measured in our study including tobacco use [40], viral STI (human

papillomavirus and herpes simplex virus-2) [40], intravaginal cleansing practices [41], and menstrual hygiene [42]. As these factors may be associated with the cervicovaginal microbiota, we cannot exclude residual or unmeasured confounding. Genital swabs were self-collected by participants, raising the potential for false negative genital swabs. In future work, β-globin PCR could be implemented as a positive control to confirm the presence of human DNA [16]. Lastly, we defined FGS by *Schistosoma* DNA detection on qPCR, however we cannot exclude that cervicovaginal qPCR detected *S. haematobium* eggs from a sexual partner's semen [16].

In conclusion, we report weak evidence of an association between *T. vaginalis* presence and FGS, with a consistently stronger association in women with a higher burden FGS infection. Additional research is needed to understand the interactions between *S. haematobium* and *T. vaginalis*, especially regarding HIV-1 vulnerability.

Footnotes

Conflict of Interest Statement:

The authors report no conflicts of interest

Funding:

Dr A Bustinduy received funding from the Wellcome Trust (Award 205954/Z/17/Z). Dr P Cools received funding from the Research Foundation – Flanders (BOFSTG2019010101) and was financially supported by a Bill and Melinda Gates Foundation Project (OPP1120972). Dr E Webb and Professor R Hayes received funding from MRC Grant Reference MR/K012126/1, and Dr SC Francis received salary from MRC Grant Reference MR/N023692/1. These awards are jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and are also part of the EDCTP2 program supported by the European Union. HPTN 071 (PopART) was supported by the National Institute of Allergy and Infectious Diseases (NIAID) under Cooperative Agreements UM1-AI068619, UM1-AI068617, and UM1-AI068613, with funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR); the International Initiative for Impact Evaluation with support from the Bill and Melinda Gates Foundation; the NIAID, the National Institute on Drug Abuse, and the National Institute of Mental Health, all part of the National Institutes of Health. Professor Eyrun Kjetland was supported by South-Eastern Regional Health Authority, Norway project #2016055. Bruno Levecke received funding from the Fund for Scientific Research-Flanders (grant no 1285316N).

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This work has not been previously presented

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Acknowledgements:

We wish to acknowledge the participants for devoting their time and trust to this study. We would like to recognize the work performed by the BILHIV ?project supervisor Namakau Chola, and the BILHIV community workers Ethel Mwansa, Mwiingana Lukonga, Ruth Mwanza, Mervis Kantukaleza, and Judith Lungu. Our work in Livingstone would not have been possible without support from Clement Mwakamui (Zambart). We gratefully acknowledge Eric A.T. Brienen (LUMC) for performing the genital *Schistosoma* PCR analysis and Claudia J. de Dood (LUMC) and Pytsje T. Hoekstra (LUMC) for performing the CAA analysis.

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REFERENCES

1. Kjetland EF, Ndhlovu PD, Gomo E, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS **2006**; 20:593-600.
2. Sturt AS, Webb EL, Francis SC, Hayes RJ, Bustinduy AL. Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition. Acta Trop **2020**; 209:105524.
3. Boris S, Barbes C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect **2000**; 2:543-6.
4. McKinnon LR, Achilles SL, Bradshaw CS, et al. The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. AIDS Res Hum Retroviruses **2019**; 35:219-28.
5. van de Wijgert J, Jespers V. The global health impact of vaginal dysbiosis. Res Microbiol **2017**; 168:859-64.
6. Torrone EA, Morrison CS, Chen PL, et al. Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. PLoS Med **2018**; 15:e1002511.
7. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. N Engl J Med **1995**; 333:1737-42.
8. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet **2015**; 385:430-40.
9. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS **2008**; 22:1493-501.
10. Jespers V, Crucitti T, Menten J, et al. Prevalence and correlates of bacterial vaginosis in different sub-populations of women in sub-Saharan Africa: a cross-sectional study. PloS one **2014**; 9:e109670.
11. Bullington BW, Lee MH, Mlingi J, et al. Cervicovaginal bacterial communities in reproductive-aged Tanzanian women with Schistosoma mansoni, Schistosoma haematobium, or without schistosome infection. ISME J **2021**.
12. Pillay P, van Lieshout L, Taylor M, et al. Cervical cytology as a diagnostic tool for female genital schistosomiasis: Correlation to cervical atypia and Schistosoma polymerase chain reaction. CytoJournal **2016**; 13:10.
13. Kjetland EF, Hove RJ, Gomo E, et al. Schistosomiasis PCR in vaginal lavage as an indicator of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2009**; 81:1050-5.
14. Galappaththi-Arachchige HN, Holmen S, Koukounari A, et al. Evaluating diagnostic indicators of urogenital Schistosoma haematobium infection in young women: A cross sectional study in rural South Africa. PloS one **2018**; 13:e0191459.
15. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, et al. Gynecological manifestations, histopathological findings, and schistosoma-specific polymerase chain reaction results among women with Schistosoma haematobium infection: a cross-sectional study in Madagascar. The Journal of infectious diseases **2015**; 212:275-84.
16. Sturt AS, Webb EL, Phiri CR, et al. Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. PLoS Negl Trop Dis **2020**; 14:e0008337.

17. Hayes RJ, Donnell D, Floyd S, et al. Effect of Universal Testing and Treatment on HIV Incidence - HPTN 071 (PopART). N Engl J Med **2019**; 381:207-18.
18. World Health Organization. Female genital schistosomiasis: a pocket atlas for clinical health-care professionals. World Health Organization. 2015. Available at: http://www.who.int/iris/handle/10665/180863 Accessed April 30, 2021.
19. Zambian Ministry of Health. Guidelines for the Etiological and Clinical Management of Sexually Transmitted Infections in Zambia, 2017.
20. 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**; 141:1841-55.
21. Corstjens P, de Dood CJ, Knopp S, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Schistosoma Infections-Improvement and Use during SCORE. Am J Trop Med Hyg **2020**; 103:50-7 doi:10.4269/ajtmh.19-0819.
22. Obeng BB, Aryeetey YA, de Dood CJ, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. Annals of tropical medicine and parasitology **2008**; 102:625-33.
23. Pillay P, Taylor M, Zulu SG, et al. Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. Am J Trop Med Hyg **2014**; 90:546-52.
24. Jespers V, van de Wijgert J, Cools P, et al. The significance of Lactobacillus crispatus and L. vaginalis for vaginal health and the negative effect of recent sex: a cross-sectional descriptive study across groups of African women. BMC Infect Dis **2015**; 15:115.
25. Sturt AS Webb EL Patterson C et al. Cervicovaginal Immune Activation in Zambian Women with Female Genital Schistosomiasis. Front Immunol (in press) **2021**.
26. Srinivasan S, Liu C, Mitchell CM, et al. Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. PloS one **2010**; 5:e10197.
27. Jarosik GP, Land CB, Duhon P, Chandler R, Jr., Mercer T. Acquisition of iron by Gardnerella vaginalis. Infect Immun **1998**; 66:5041-7.
28. Jourdan PM, Roald B, Poggensee G, Gundersen SG, Kjetland EF. Increased vascularity in cervicovaginal mucosa with Schistosoma haematobium infection. PLoS Negl Trop Dis **2011**; 5:e1170.
29. Vaneechoutte M. Lactobacillus iners, the unusual suspect. Res Microbiol **2017**; 168:826-36.
30. Nunn KL, Wang YY, Harit D, et al. Enhanced Trapping of HIV-1 by Human Cervicovaginal Mucus Is Associated with Lactobacillus crispatus-Dominant Microbiota. mBio **2015**; 6:e01084-15.
31. Kleppa E, Ramsuran V, Zulu S, et al. Effect of female genital schistosomiasis and anti-schistosomal treatment on monocytes, CD4+ T-cells and CCR5 expression in the female genital tract. PloS one **2014**; 9:e98593.
32. Crucitti T, Jespers V, Mulenga C, Khondowe S, Vandepitte J, Buve A. Trichomonas vaginalis is highly prevalent in adolescent girls, pregnant women, and commercial sex workers in Ndola, Zambia. Sex Transm Dis **2010**; 37:223-7.
33. Hotez PJ, Kamath A. Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. PLoS Negl Trop Dis **2009**; 3:e412.

34. Rowley J, Vander Hoorn S, Korenromp E, et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ **2019**; 97:548-62P.
35. Cortes-Selva D, Fairfax K. Schistosome and intestinal helminth modulation of macrophage immunometabolism. Immunology **2021**; 162:123-34.
36. Han IH, Song HO, Ryu JS. IL-6 produced by prostate epithelial cells stimulated with Trichomonas vaginalis promotes proliferation of prostate cancer cells by inducing M2 polarization of THP-1-derived macrophages. PLoS Negl Trop Dis **2020**; 14:e0008126.
37. Hsieh YJ, Fu CL, Hsieh MH. Helminth-induced interleukin-4 abrogates invariant natural killer T cell activation-associated clearance of bacterial infection. Infect Immun **2014**; 82:2087-97.
38. Bharti R, Grimm DG. Current challenges and best-practice protocols for microbiome analysis. Brief Bioinform **2021**; 22:178-93.
39. Jo JH, Kennedy EA, Kong HH. Research Techniques Made Simple: Bacterial 16S Ribosomal RNA Gene Sequencing in Cutaneous Research. J Invest Dermatol **2016**; 136:e23-e7.
40. Cherpes TL, Hillier SL, Meyn LA, Busch JL, Krohn MA. A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. Sex Transm Dis **2008**; 35:78-83.
41. Low N, Chersich MF, Schmidlin K, et al. Intravaginal practices, bacterial vaginosis, and HIV infection in women: individual participant data meta-analysis. PLoS Med **2011**; 8:e1000416.
42. Das P, Baker KK, Dutta A, et al. Menstrual Hygiene Practices, WASH Access and the Risk of Urogenital Infection in Women from Odisha, India. PloS one **2015**; 10:e0130777.

Table 5.1 Baseline characteristics of the study population (n=213) by female genital schistosomiasis status

Participant Characteristics		FGS Negative (n)% (n=158)	Probable FGS (n)% (n=25)	FGS (n)% (n=30)	p-value*
Age in years – Median(IQR)		23 (22 – 24)	27 (23 – 31)	22 (21 – 24)	0.002‡
Marital status	Single	72 (45.6)	4 (16.0)	13 (43.)	0.04
	Married or Cohabitating	81 (51.3)	20 (80.0)	17 (56.7)	
	Divorced, or Separated	5 (3.2)	1 (4.0)	0 (0.0)	
Education (highest level)	None or Any Primary School	35 (22.2)	12 (48.0)	10 (33.3 (10)	0.06
	Any Secondary School	1111 (70.3)	13 (52.0)	19 (63.3)	
	Trade, Degree or higher	12 (7.6)	0 (0.0)	1 (3.3)	
District	Community A	66 (41.8)	20 (80.0)	22 (73.3)	<0.01
	Community B	92 (58.2)	5 (20.0)	8 (26.7)	
Household members	1-3	51 (32.3)	4 (16.0)	12 (40.0)	0.3
	4-5	61 (38.6)	13 (52.0)	8 (26.7)	
	6+	46 (29.1)	8 (32.0)	10 (33.3)	
Employment status	Not Working	117 (74.1)	14 (56.0)	26 (86.7)	0.04
	Working	41 (25.9)	11 (44.0)	4 (13.3)	
Sexual behaviour characteristics					
Age at sexual debut (years)	8-16	64 (40.5)	11 (44.0)	17 (56.7)	0.4
	17-19	74 (46.8)	13 (52.0)	10 (33.3)	
	20-24	20 (12.7)	1 (4.0)	3 (10.0)	
Lifetime sexual partners	1	57 (36.1)	8 (32.0)	5 (16.7)	0.3
	2	36 (22.8)	6 (24.0)	9 (30.0)	
	3	23 (14.6)	6 (24.0)	8 (26.7)	
	4+	42 (26.6)	5 (20.0)	8 (26.7)	
Currently sexually active**,†	No	26 (16.6)	2 (8.0)	3 (10.0)	0.5
	Yes	131 (83.4)	23 (92.0)	27 (90.0)	
Condom use with last sex††	No	120 (76.9)	16 (66.7)	22 (73.3)	0.6‡‡
	Yes	36 (23.1)	8 (33.3)	8 (26.7)	
HIV-1 Status	Not Detected	132 (83.5)	20 (80.0)	24 (80.0)	0.8
	Detected	26 (16.5)	5 (20.0)	6 (20.0)	
Any STI***	Not Detected	106 (67.1)	14 (56.0)	18 (60.0)	0.5
	Detected	52 (32.9)	11 (44.0)	12 (40.0)	
Contraceptive Use					
Condoms	No	132 (83.5)	20 (80.0)	26 (86.7)	0.8
	Yes	26 (16.5)	5 (20.0)	4 (13.3)	
OCP	No	148 (93.7)	22 (88.0)	29 (96.7)	0.5
	Yes	10 (6.3)	3 (12.0)	1 (3.3)	
Injectable	No	82 (51.9)	13 (52.0)	15 (50.0)	1.0‡‡
	Yes	76 (48.1)	12 (48.0)	15 (50.0)	
Implant	No	144 (91.1)	23 (92.0)	28 (93.3)	1.0
	Yes	14 (8.9)	2 (8.0)	2 (6.7)	
Any Hormonal Contraception~	No	58 (36.7)	8 (32.0)	10 (35.7)	0.9‡‡
	Yes	100 (63.3)	17 (68.0)	18 (64.3)	
Schistosomiasis-related Factors					
Urine microscopy	Not Detected	158 (100.0)	19 (76.0)	11 (36.7)	<0.001ϕϕ
	Detected	0 (0.0)	6 (24.0)	19 (63.3)	
Urine CAA	Negative	158 (100.0)	0 (0.0)	11 (36.7)	<0.001ϕϕ
	Positive	0 (0.0)	25 (100.0)	19 (63.3)	
Active Infection~~	Not Present	158 (100.0)	0 (0.0)	9 (30.0)	<0.001ϕϕ
	Present	0 (0.0)	25 (100.0)	21 (70.0)	

*Fisher's exact p-value unless otherwise indicated

‡ Kruskal Wallis p-value
‡‡ Chi squared p-value
** Any sexual activity in the last 6 months
† Participants who responded with "no answer" (n=1) are not shown in the table
†† Participants who responded with "no answer" (n=3) are not shown in the table
*** Any STI defined as the presence of at least one of *N. gonorrhoeae, C. trachomatis, M. genitalium* or *T. vaginalis*
˜ Any hormonal contraception is defined as use of injectable agents, implants, or oral contraceptive pills
~~ Active infection defined as the presence of either a positive CAA or urine microscopy for *S. haematobium* eggs, or both
ϕϕ Part of the definition for FGS categories

Table 5.2 – Presence and concentration of vaginal *Lactobacilli*, other key microbiota, and sexually transmitted infection, overall and by female genital schistosomiasis status

Organism	n(%) and Concentration*	All participants (n=213)	FGS Negative (n=158)	FGS (n=30)	p-value**
L. crispatus	Presence	69 (32.4)	49 (31.0)	13 (43.3)	0.19
	Median (IQR)	$8.7x10^6$ ($2.6x10^5$ – $4.2x10^8$)	$1.1x10^7$ ($2.6x10^5$ – $7.0x10^8$)	$7.5x10^5$ ($1.9x10^5$ – $2.7x10^7$)	0.20
	Log concentration mean	16.01	16.38	14.82	0.19
L. iners	Presence	156 (73.2)	113 (71.5)	24 (80.0)	0.34
	Median (IQR)	$2.7x10^8$ ($3.8x10^7$ – $1.5x10^9$)	$2.8x10^8$ ($3.9x10^7$ – $1.6x10^9$)	$1.7x10^8$ ($1.6x10^7$ – $1.0x10^9$)	0.39
	Log concentration mean	18.93	18.98	18.36	0.33
G. vaginalis	Presence	156 (73.2)	115 (72.8)	23 (76.7)	0.66
	Median (IQR)	$7.7x10^6$ ($8.3x10^5$ – $5.2x10^7$)	$8.1x10^6$ ($7.7x10^5$ – $4.8x10^7$)	$3.8x10^6$ ($6.8x10^5$ – $6.4x10^7$)	0.93
	Log concentration mean	15.85	15.74	15.91	0.79
A. vaginae	Presence	152 (71.4)	112 (70.9)	19 (63.3)	0.41
	Median (IQR)	$5.8x10^7$ ($8.2x10^6$ – $2.1x10^8$)	$5.8x10^7$ ($8.7x10^6$ – $2.0x10^8$)	$3.0x10^7$ ($1.06x10^6$ – $2.8x10^8$)	0.83
	Log concentration mean	17.31	17.33	17.06	0.66
T. vaginalis	Presence	53 (24.9)	34 (21.5)	11 (36.7)	0.08
	Median (IQR)	$4.2x10^4$ (173.0 – $2.3x10^6$)	$1.7x10^5$ (56.9 – $6.3x10^6$)	$1.0x10^4$ (5030.0 – $4.5x10^5$)	0.53
	Log concentration mean	10.39	10.88	10.19	0.69
Vaginal Microbiota with prevalence <20%					
N. gonorrhoeae	Presence	13 (6.1)	12 (7.6)	0.0 (0)	0.22
C. trachomatis	Presence	17 (8.0)	13 (8.2)	3 (10.0)	0.72
M. genitalium	Presence	8 (3.8)	7 (4.4)	0 (0.0)	0.60
Candida spp.	Presence	12 (5.63)	8 (5.1)	2 (6.7)	0.66

*concentrations are expressed in genome equivalents/mL;

** For species with >20% prevalence, p-values for presence, median (IQR) and log concentration mean from chi-squared test, ranksum test and t-test, respectively. For species with <20% prevalence, p-values for presence are from Fisher's exact test.

Table 5.3 – Ad-hoc analysis of the presence and concentration of vaginal *Lactobacilli*, other cervicovaginal microbiota, and sexually transmitted infection in participants with moderate to high concentration of *Schistosoma* DNA (Ct <35) compared to female genital schistosomiasis negative participants

Organism	n(%) and Concentration*	FGS Negative (n=158)	FGS with Ct<35 (n=16)	p-value**
L. crispatus	Presence	49 (31.0)	6 (37.5)	0.60
	Median (IQR)	$1.1x10^7$ ($2.6x10^5$ – $7.0x10^8$)	$1.2x10^6$ ($3.0x10^5$ – $2.0x10^7$)	0.28
	Log concentration mean	16.38	14.27	0.19
L. iners	Presence	113 (71.5)	11 (68.75)	0.82
	Median (IQR)	$2.8x10^8$ ($3.9x10^7$ – $1.6x10^9$)	$7.5x10^7$ ($3.6x10^6$ – $7.5x10^8$)	0.30
	Log concentration mean	18.98	17.86	0.21
G. vaginalis	Presence	115 (72.8)	13 (81.3)	0.46
	Median (IQR)	$8.1x10^6$ ($7.7x10^5$ – $4.8x10^7$)	$1.5x10^7$ ($3.8x10^6$ – $3.1x10^8$)	0.11
	Log concentration mean	15.74	17.45	0.03
A. vaginae	Presence	112 (70.9)	13 (81.3)	0.38
	Median (IQR)	$5.8x10^7$ ($8.7x10^6$ – $2.0x10^8$)	$1.6x10^8$ ($1.6x10^7$ – $2.8x10^8$)	0.70
	Log concentration mean	17.33	17.38	0.95
T. vaginalis	Presence	34 (21.5)	8 (50.0)	0.01
	Median (IQR)	$1.7x10^5$ (56.9 – $6.3x10^6$)	8085.0 (5210.0 – $1.0x10^6$)	0.56
	Log concentration mean	10.88	10.09	0.70
Vaginal Microbiota with Prevalence <20%				
N. gonorrhoeae	Presence	12 (7.6)	0 (0.0)	0.61
C. trachomatis	Presence	13 (8.2)	2 (12.5)	0.63
M. genitalium	Presence	7 (4.4)	0 (0.0)	1.0
Candida spp.	Presence	8 (5.1)	2 (12.5)	0.23

*concentrations are expressed in genome equivalents/mL

** For species with >20% prevalence, p-values for presence, median (IQR) and log concentration mean from chi-squared test, ranksum test and t-test, respectively. For species with <20% prevalence, p-values for presence are from Fisher's exact test.

Table 5.4 – Ad-hoc analysis of the presence and concentration of vaginal *Lactobacilli*, cervicovaginal microbiota, and sexually transmitted infection in participants with *Schistosoma* DNA detected in ≥ 2 genital specimens compared to female genital schistosomiasis negative participants

Organism	n(%) and Concentration*	FGS Negative (n=158)	≥ 2 PCR FGS (n=16)	p-value**
L. crispatus	Presence	49 (31.0)	7 (43.8)	0.30
	Median (IQR)	$1.1x10^7$ ($2.6x10^5$ – $7.0x10^8$)	$1.9x10^6$ ($7.7x10^4$ – $2.7x10^7$)	0.32
	Log concentration mean	16.38	14.90	0.34
L. iners	Presence	113 (71.5)	10 (62.5)	0.45
	Median (IQR)	$2.8x10^8$ ($3.9x10^7$ – $1.6x10^9$)	$5.1x10^7$ ($9.0x10^5$ – $1.1x10^8$)	0.03
	Log concentration mean	18.98	17.00	0.03
G. vaginalis	Presence	115 (72.8)	14 (87.5)	0.20
	Median (IQR)	$8.1x10^6$ ($7.7x10^5$ – $4.8x10^7$)	$1.6x10^7$ ($1.1x10^6$ – $3.6x10^8$)	0.29
	Log concentration mean	15.74	16.85	0.16
A. vaginae	Presence	112 (70.9)	11 (68.8)	0.86
	Median (IQR)	$5.8x10^7$ ($8.7x10^6$ – $2.0x10^8$)	$1.6x10^8$ ($2.7x10^7$ – $3.2x10^8$)	0.33
	Log concentration mean	17.33	18.04	0.36
T. vaginalis	Presence	34 (21.5)	8 (50.0)	0.01
	Median (IQR)	$1.7x10^5$ (56.9 – $6.3x10^6$)	5680.0 (2967.5 – $1.0x10^6$)	0.50
	Log concentration mean	10.88	9.71	0.56
Vaginal Microbiota with Prevalence <20%				
N. gonorrhoeae	Presence	12 (7.6)	0 (0.0)	0.61
C. trachomatis	Presence	13 (8.2)	2 (12.5)	0.63
M. genitalium	Presence	7 (4.4)	0 (0.0)	1.0
Candida spp.	Presence	8 (5.1)	1 (6.3)	0.59

*concentrations are expressed in genome equivalents/mL

** For species with >20% prevalence, p-values for presence, median (IQR) and log concentration mean from chi-squared test, ranksum test and t-test, respectively. For species with <20% prevalence, p-values for presence are from Fisher's exact test.

Figure 5.1 – Stacked bar chart of concentrations of cervicovaginal microbiota and taxa causing sexually tract infections by female genital schistosomiasis status

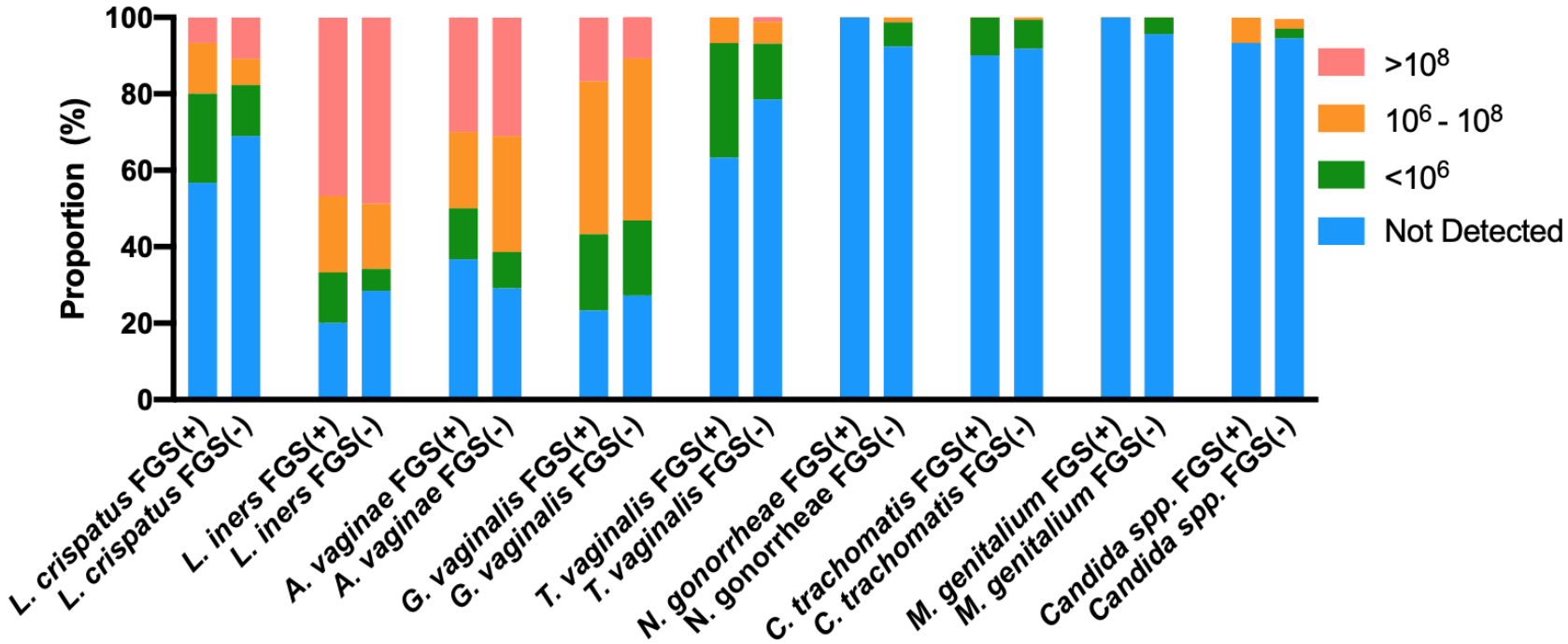


Table 5.S1 – Crude and adjusted associations of presence and mean log-transformed concentrations of vaginal *Lactobacilli*, other cervicovaginal microbiota, and sexually transmitted infection with female genital schistosomiasis status

Organism	n(%) and Concentration*	All Participants (n=213)	FGS Negative (n=158)	FGS (n=30)	Crude Regression Estimate** (OR, difference in means)	p-value ††	Adjusted Regression Estimate††† (OR, difference in means)	LRT p-value
L. crispatus	Presence**	69 (32.4)	49 (31.0)	13 (43.3)	1.70 (0.77 – 3.77)	0.19	1.68 (0.74 – 3.79)	0.22
	Log conc mean †	16.01	16.38	14.82	-1.56 (-3.90 – 0.78)	0.19	-1.93 (-4.57 – 0.71)	0.13
L. iners	Presence**	156 (73.2)	113 (71.5)	24 (80.0)	1.60 (0.61 – 4.16)	0.34	1.68 (0.74 – 3.79)	0.22
	Log conc mean †	18.93	18.98	18.36	-0.62 (-1.88 – 0.64)	0.33	-0.53 (-1.83 – 0.77)	0.41
G. vaginalis	Presence**	156 (73.2)	115 (72.8)	23 (76.7)	1.23 (0.49 – 3.07)	0.66	1.22 (0.49 – 3.05)	0.67
	Log conc mean †	15.85	15.74	15.91	-0.17 (-1.08 – 1.41)	0.79	0.24 (-1.05 – 1.53)	0.71
A. vaginae	Presence**	152 (71.4)	112 (70.9)	19 (63.3)	0.71 (0.31 – 1.61)	0.41	0.71 (0.31 – 1.61)	0.42
	Log conc mean †	17.31	17.33	17.06	-0.27 (-1.51 – 0.96)	0.66	-0.18 (-1.44 – 1.07)	0.77
T. vaginalis	Presence**	53 (24.9)	34 (21.5)	11 (36.7)	2.11 (0.92 – 4.86)	0.08	2.09 (0.90 – 4.83)	0.09
	Log conc mean †	10.39	10.88	10.19	-0.69 (-4.16 – 2.77)	0.69	-0.65 (-4.47 – 3.17)	0.71
Vaginal microbiota with prevalence <20%								
N. gonorrhoeae	Presence	13 (6.1)	12 (7.6)	0.0 (0)	Not applicable	0.22	Not applicable	NA
C. trachomatis	Presence**	17 (8.0)	13 (8.2)	3 (10.0)	1.24 (0.33 – 4.64)	0.72	1.20 (0.32 – 4.54)	0.79
M. genitalium	Presence	8 (3.8)	7 (4.4)	0 (0.0)	Not applicable	0.60	Not applicable	NA
Candida spp.	Presence**	12 (5.63)	8 (5.1)	2 (6.7)	1.34 (0.27 – 6.64)	0.66	1.33 (0.27 – 6.61)	0.73

*concentrations are expressed in genome equivalents/mL

** for presence/absence, regression estimates are odds ratios (OR) generated by logistic regression, for log concentration mean, regression estimates are differences in means generated by linear regression

†† For species with >20% prevalence, p-values for presence and log concentration mean are from chi-squared test and t-test, respectively. For species with <20% prevalence, p-values for presence are from Fisher's exact test.

†††presence/absence logistic regression adjusted for age, log concentration mean linear regression adjusted for age, community of residence and education

Table 5.S2 – Presence and mean log-transformed concentrations of vaginal Lactobacilli, other cervicovaginal microbiota, and sexually transmitted infection with crude and adjusted associations comparing participants with FGS or probable FGS (combined) with those who were FGS negative

Organism	n(%) and Concentration*	All Participants (n=213)	FGS Negative (n=158)	FGS & Probable FGS (n=55)	p-value**
L. crispatus	Presence	69 (32.4)	49 (31.0)	20 (36.4)	0.47
	Median (IQR)	$8.7x10^6$ ($2.6x10^5$ – $4.2x10^8$)	$1.1x10^7$ ($2.6x10^5$ – $7.0x10^8$)	$1.3x10^6$ ($2.3x10^5$ – $2.3x10^7$)	0.24
	Log concentration mean	16.01	16.38	15.11	0.21
L. iners	Presence	156 (73.2)	113 (71.5)	43 (78.2)	0.34
	Median (IQR)	$2.7x10^8$ ($3.8x10^7$ – $1.5x10^9$)	$2.8x10^8$ ($3.9x10^7$ – $1.6x10^9$)	$2.7x10^8$ ($3.3x10^7$ – $1.4x10^9$)	0.73
	Log concentration mean	18.93	18.98	18.81	0.74
G. vaginalis	Presence	156 (73.2)	115 (72.8)	41 (74.6)	0.80
	Median (IQR)	$7.7x10^6$ ($8.3x10^5$ – $5.2x10^7$)	$8.1x10^6$ ($7.7x10^5$ – $4.8x10^7$)	$5.9x10^6$ ($1.1x10^6$ – $1.0x10^8$)	0.52
	Log concentration mean	15.85	15.74	16.14	0.42
A. vaginae	Presence	152 (71.4)	112 (70.9)	40 (72.7)	0.79
	Median (IQR)	$5.8x10^7$ ($8.2x10^6$ – $2.1x10^8$)	$5.8x10^7$ ($8.7x10^6$ – $2.0x10^8$)	$6.1x10^7$ ($4.7x10^6$ – $2.7x10^8$)	0.97
	Log concentration mean	17.31	17.33	17.25	0.87
T. vaginalis	Presence	53 (24.9)	34 (21.5)	19 (34.6)	0.05
	Median (IQR)	$4.2x10^4$ (173.0 – $2.3x10^6$)	$1.7x10^5$ (56.9 – $6.3x10^6$)	$6.0x10^3$ (361.0 – $4.5x10^5$)	0.35
	Log concentration mean	10.39	10.88	9.52	0.35
Vaginal microbiota with prevalence <20%					
N. gonorrhoeae	Presence	13 (6.1)	12 (7.6)	1 (1.8)	0.19
C. trachomatis	Presence	17 (8.0)	13 (8.2)	4 (7.3)	1.0
M. genitalium	Presence	8 (3.8)	7 (4.4)	1 (1.8)	0.68
Candida spp.	Presence	12 (5.63)	8 (5.1)	4 (7.3)	0.51

*concentrations are expressed in genome equivalents/mL

** For species with >20% prevalence, p-values for presence, median (IQR) and log concentration mean from chi-squared test, ranksum test and t-test, respectively. For species with <20% prevalence, p-values for presence are from Fisher's exact test.

Figure 5.S1 – Causal diagram describing the association between FGS and a concentration change of cytokines and chemokines

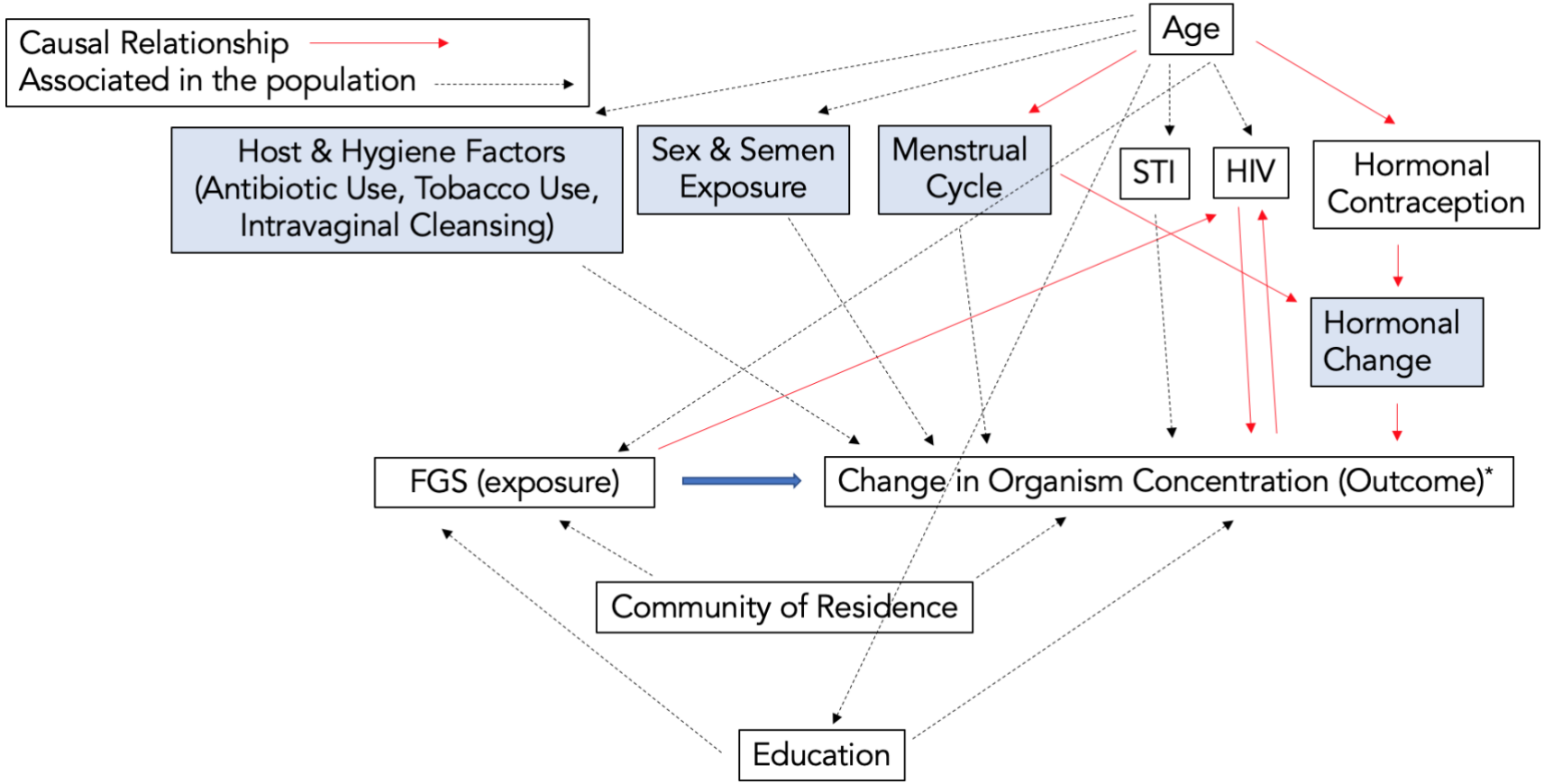
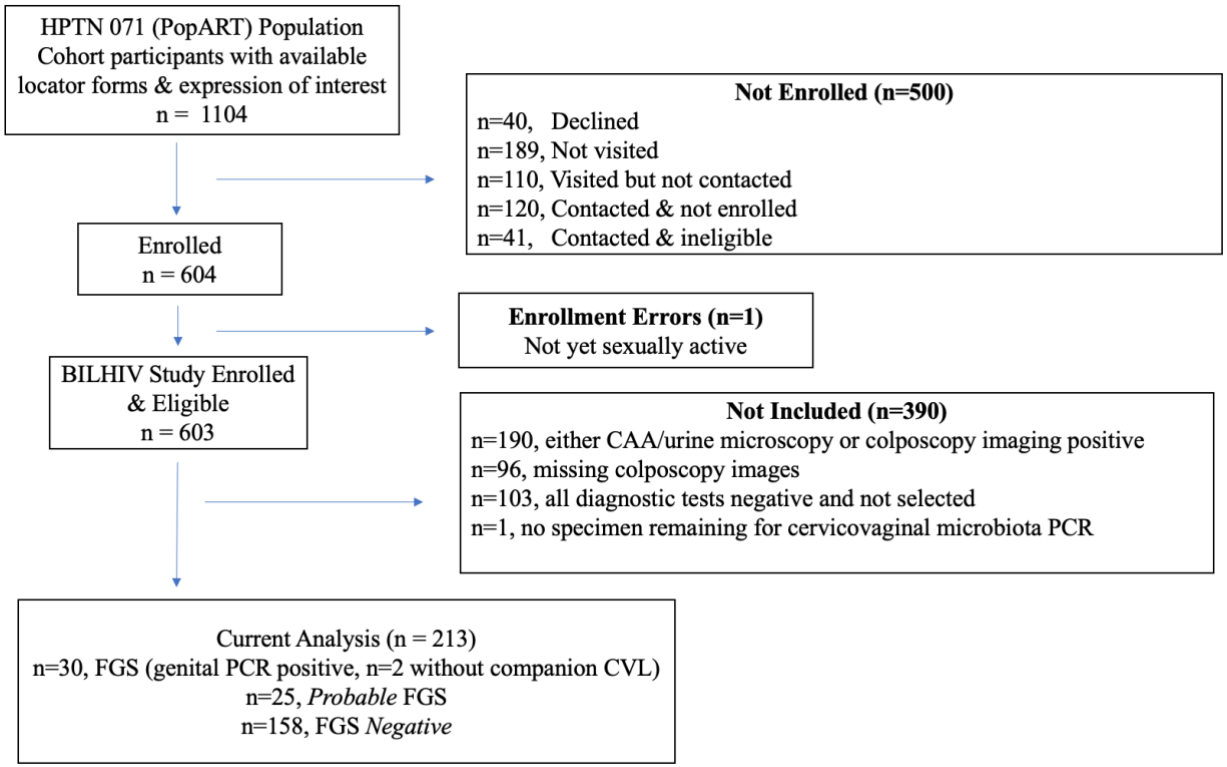


Figure 5.S2 – Study flow diagram



Further details of home and clinic-based sample collection

This section provides additional detail to the details of home and clinic-based sample collection provided in the main manuscript.

The BILHIV study home visit included written informed consent, a questionnaire, genital self-sampling (cervical and vaginal), and collection of a urine specimen, as previously described [1]. Specimens were placed in cool boxes for transportation and were stored in the laboratory at -80°C. Vaginal and cervical swab specimens were used for PCR detection of *Schistosoma* species; cervical swabs were used for characterization of the microbiota and STI by quantitative PCR (qPCR); urine was used for detection of circulating anodic antigen (CAA) and microscopic evaluation of the urine for *S. haematobium* eggs.

Enrolled women who were not currently menstruating were invited to attend Livingstone Central Hospital cervical cancer screening clinic, where one of two trained midwives performed cervicovaginal lavage (CVL), which was used for *Schistosoma* PCR. After speculum insertion, a bulb syringe was used to flush normal saline (10 mL) continuously across the cervix and vaginal walls for one minute. CVL fluid was collected from the posterior fornices and stored temporarily in a refrigerator (4 °C) on ice until transfer to the laboratory, where specimens were stored at -80°C. All specimens were shipped on dry ice to Leiden University Medical Center (LUMC).

Cervicovaginal images were captured with a portable colposcope (MobileODT, Tel Aviv, Israel) and were evaluated by one author (EFK) for the presence of any of the four recognized FGS cervicovaginal manifestations: grainy sandy patches, homogenous yellow sandy patches, rubbery papules, and abnormal blood vessels [2]. Women with at least one of these manifestations [2] and

women with any positive urine or genital *Schistosoma* diagnostic were treated free of charge with 40 mg/kg praziquantel. Testing for STI was not performed at the point-of-care and participants with suspected STI were offered syndromic management, as per local guidelines [3].

Urine microscopy and Circulating Anodic Antigen

After aliquoting for CAA quantification, urine was centrifuged in 15 mL aliquots and examined by microscopy for *S. haematobium* eggs within 24 hours. The participant was considered positive if a pellet contained at least one *S. haematobium* egg, as previously described [1]. A lateral flow assay utilizing up-converting reporter particles for the quantification of CAA was performed on urine samples, as previously described [1, 4]. CAA levels reflect the burden of live schistosomes and decline after successful treatment with praziquantel [5, 6]. Analyzing the equivalent of 417 µL urine (wet reagent, UCAA***hT***417), a test result indicating a CAA value of >0.6 pg/mL was considered positive [6].

PCR for detection of *Schistosoma* DNA

DNA extraction and detection of the *Schistosoma*-specific internal-transcribed-spacer-2 (ITS2) target by real-time PCR were performed at LUMC, using a custom automated liquid handling station (Hamilton, Switzerland), as previously described [1, 7]. DNA extraction of 200 µL of CVL, cervical or vaginal swab fluid was done with QIAamp spin columns (QIAGEN, Benelux; Venlo, The Netherlands) according to the manufacturer's guidelines. Schistosome DNA amplification and detection were performed with the CFX96 Real Time PCR Detection System and BioRad CFX software (BioRad, California, USA). DNA extracted from cervical swabs was transported to Ghent University for further analysis.

Cervicovaginal microbiota characterization and STI detection

DNA from cervical swabs were used to quantified key markers of vaginal health (*Lactobacillus crispatus*), BV (*Gardnerella vaginalis* and *Atopobium vaginae*), *Lactobacillus iners* (a highly prevalent lactobacillus with an enigmatic role), *Candida* spp. and STI (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis)* by means of qPCR at the Laboratory Bacteriology Research (Ghent University, Ghent, Belgium).

For the *L. crispatus*, *L. iners*, *G. vaginalis*, *A. vaginae* and *Candida* spp qPCR, a total reaction volume of 10 µL was prepared by the addition of 2 µL of DNA extract of the CVL, positive control (i.e., DNA of the corresponding type strain (listed for each species in Table 1)) or negative control (HPLC water) to 8 µL of reaction mixture. The final reaction mixture of each qPCR consisted of primers (listed in Table 2) in 1X LightCycler 480 SYBR Green I master mix in HPLC water. Reaction conditions for *A. vaginae* were pre-incubation for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 20 s at 62 °C and 40 s at 72 °C, for *G. vaginalis* pre-incubation 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 56 °C and 30 s at 72 °C, for *L. crispatus* pre-incubation for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, for *L. iners* pre-incubation for 10 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 20 s at 50 °C and 4 s at 72 °C and *Candida* spp. by pre-incubation for 10 min at 95 °C, followed by 45 cycles of 20 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C. High resolution melting curves were generated for each species by melting all amplified double stranded DNA at 95 °C for 5 s, followed by renaturating DNA for 30 s at 50 °C (*A. vaginae*), 60 s at 55 °C (*G. vaginalis*), 60 s at 60 °C (*L. crispatus*, *L. iners* and *Candida* spp.), whereafter the temperature was increased to 97 °C at a ramp

rate of 0.02 °C per s. *Candida* speciation was determined based on the melting peak temperatures as previously described [8].

We quantified *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and *T. vaginalis* using the S-DiaCTNG™ (for *C. trachomatis* and *N. gonorrhea*) and S-DiaMGTV™ (for *M. genitalium* and *T. vaginalis*) (both Diagenode Diagnostics, Seraing, Belgium) according to the manufacturer's instructions.

To quantify each of the target species, standard curves were constructed from a tenfold dilution series of DNA from *C. trachomatis* (ATCC VR-571B), *N. gonorrhoeae*, *M. genitalium* (ATCC G37), and *T. vaginalis* (ATCC 50148) (all commercially purchased). Genomic DNA from *N. gonorrhoeae* (ATCC 43069), *A. vaginae*, *G. vaginalis*, *L. crispatus*, *L. iners* and *C. albicans* was obtained after culturing the strains according to conditions from Table 1 and extracting DNA from colonies using Roche High Pure DNA Purification kit (Roche). All DNA concentrations were determined using NanoDrop (Thermo Fisher scientific, Erembodegem, Belgium). The genomic concentrations were calculated using the described genomic sizes of the type strains. Both the standard curves and samples were run in duplicate. The bacterial, fungal and protozoan concentrations were expressed as genome equivalents per mL (ge/mL) [9].

All non-schistosome qPCR assays were performed using the LightCycler480® and the LightCyclerR 480 Software Version 1.5 (Roche, Basel, Switzerland).

Table 1: Strains used in this study

Species	Strain	Culture conditions
Atopobium vaginae	CCUG 38953^T	Anaerobe, 37 °C, TSA plates
Gardnerella vaginalis	LMG 7832^T	Anaerobe, 37 °C, chocolate agar plates

Lactobacillus crispatus	LMG 9479^{T}	Anaerobe, 37 °C, NYC+ HS agar plates
Lactobacillus iners	ACS-049-V-Sch2	Anaerobe, 37 °C, NYC+ HS agar plates
Candida albicans	ATCC 90028	Aerobe, 30 °C, CHROMID® Candida agar
Chlamydia trachomatis	ATCC VR-571B	N/A
Neisseria gonorrhoeae	ATCC 43069	35°C ±1°C for 5 days on chocolate agar (Becton Dickinson)
Mycoplasma genitalium	ATCC G37	N/A
Trichomonas vaginalis	ATCC 50148	N/A

Table 2: Primers used in this study

Species	Forward primer (5'-3')	Final concentration (µM)	Reverse Primer (5'-3')	Final concentration (µM)
Atopobium vaginae	CCCTATCCGCTCCTGATACC	0.7	CCAAATATCTGCGCATTTCA	0.7
Gardnerella vaginalis	TATTATAACTAAAGCTGCTG	0.5	CGCCACTATAGTCG	0.5
Lactobacillus crispatus	AGCGAGCGGAACTAACAGATTTAC	0.1	AGCTGATCATGCGATCTGCTT	0.1
Lactobacillus iners	GTCTGCCTTGAAGATCGG	0.2	ACAGTTGATAGGCATCATC	0.2
Candida spp.	GTGAATCATCGAATCTTTGAAC	0.5	TCCTCCGCTTATTGATATGC	0.5

REFERENCES

1. Sturt AS, Webb EL, Phiri CR, et al. Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. PLoS Negl Trop Dis **2020**; 14:e0008337.
2. World Health Organization. Female genital schistosomiasis: a pocket atlas for clinical health-care professionals. World Health Organization. 2015. Available at, http://www.who.int/iris/handle/10665/180863 [accessed January 12, 2021].
3. Zambian Ministry of Health. Guidelines for the Etiological and Clinical Management of Sexually Transmitted Infections in Zambia, 2017.
4. 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**; 141:1841-55.
5. van Lieshout L, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop **2000**; 77:69-80.
6. Corstjens P, de Dood CJ, Knopp S, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Schistosoma Infections-Improvement and Use during SCORE. Am J Trop Med Hyg **2020**; 103:50-7 doi:10.4269/ajtmh.19-0819.
7. Obeng BB, Aryeetey YA, de Dood CJ, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. Annals of tropical medicine and parasitology **2008**; 102:625-33.
8. Duyvejonck H, Cools P, Decruyenaere J, et al. Validation of High Resolution Melting Analysis (HRM) of the Amplified ITS2 Region for the Detection and Identification of Yeasts from Clinical Samples: Comparison with Culture and MALDI-TOF Based Identification. PloS one **2015**; 10:e0132149.
9. Jespers V, van de Wijgert J, Cools P, et al. The significance of Lactobacillus crispatus and L. vaginalis for vaginal health and the negative effect of recent sex: a cross-sectional descriptive study across groups of African women. BMC Infect Dis **2015**; 15:115.

Chapter 6

Research paper 3

Chapter 6 – Cervicovaginal immune activation in Zambian women with female genital schistosomiasis.

Citation: Sturt AS, Webb EL, Patterson C, et al. Cervicovaginal Immune Activation in Zambian Women with Female Genital Schistosomiasis. Front Immunol **2021**; 12:620657.

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ORIGINAL RESEARCH
published: 02 March 2021
doi: 10.3389/fimmu.2021.620657

Cervicovaginal Immune Activation in Zambian Women With Female Genital Schistosomiasis

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Specialty section:
This article was submitted to Microbial Immunology, a section of the journal Frontiers in Immunology

Received: *23 October 2020*
Accepted: *15 January 2021*
Published: *02 March 2021*

Citation:
Sturt AS, Webb EL, Patterson C, Phiri CR, Mweene T, Kjetland EF, Mudenda M, Mapani J, Mutengo MM, Chipeta J, van Dam GJ, Corstjens PLAM, Ayles H, Hayes RJ, Hansingo I, Cools P, van Lieshout L, Helmby H, McComsey GA, Francis SC and Bustinduy AL (2021) Cervicovaginal Immune Activation in Zambian Women With Female Genital Schistosomiasis. Front. Immunol. 12:620657. doi: 10.3389/fimmu.2021.620657

HIV-1 infection disproportionately affects women in sub-Saharan Africa, where areas of high HIV-1 prevalence and *Schistosoma haematobium* endemicity largely overlap. Female genital schistosomiasis (FGS), an inflammatory disease caused by *S. haematobium* egg deposition in the genital tract, has been associated with prevalent HIV-1 infection. Elevated levels of the chemokines MIP-1α (CCL-3), MIP-1β (CCL-4), IP-10 (CXCL-10), and IL-8 (CXCL-8) in cervicovaginal lavage (CVL) have been associated with HIV-1 acquisition. We hypothesize that levels of cervicovaginal cytokines may be raised in FGS and could provide a causal mechanism for the association between FGS and HIV-1. In the cross-sectional BILHIV study, specimens were collected from 603 female participants who were aged 18–31 years, sexually active, not pregnant and participated in the HPTN 071 (PopART) HIV-1 prevention trial in Zambia. Participants self-collected urine, and vaginal and cervical swabs, while CVLs were clinically obtained. Microscopy and *Schistosoma* circulating anodic antigen (CAA) were performed on urine. Genital samples were examined for parasite-specific DNA by PCR. Women with FGS (n=28), defined as a positive *Schistosoma* PCR from any genital sample were frequency age-matched with 159 FGS negative (defined as negative *Schistosoma* PCR, urine CAA, urine microscopy, and colposcopy imaging) women. Participants with probable FGS (n=25) (defined as the presence of either urine CAA or microscopy in combination with one of four clinical findings suggestive of FGS on colposcope-obtained photographs) were also included, for a total sample size of 212. The concentrations of 17 soluble cytokines and chemokines were quantified by a multiplex bead-based immunoassay. There was no

difference in the concentrations of cytokines or chemokines between participants with and without FGS. An exploratory analysis of those women with a higher FGS burden, defined by ≥2 genital specimens with detectable *Schistosoma* DNA (n=15) showed, after adjusting for potential confounders, a higher Th2 (IL-4, IL-5, and IL-13) and pro-inflammatory (IL-15) expression pattern in comparison to FGS negative women, with differences unlikely to be due to chance (p=0.037 for IL-4 and p<0.001 for IL-5 after adjusting for multiple testing). FGS may alter the female genital tract immune environment, but larger studies in areas of varying endemicity are needed to evaluate the association with HIV-1 vulnerability.

Keywords: HIV-1, female genital schistosomiasis, S. haematobium, inflammation, sub-Saharan Africa, genital tract, cervicovaginal lavage (CVL), polymerase chain reaction (PCR)

INTRODUCTION

HIV-1 infection disproportionately affects women in sub-Saharan Africa (1), where areas of high HIV-1 prevalence and *Schistosoma haematobium* endemicity largely overlap (2). Female genital schistosomiasis (FGS), caused most frequently by *S. haematobium* egg deposition in the genital tract, has been associated with prevalent HIV-1 infection in cross-sectional studies (3). The presence of *S. haematobium* eggs in genital tissue is also associated with vascularization (4) and the accumulation of CD4+ lymphocytes and macrophages (5), making the granuloma-associated environment a potential contributor to HIV-1 vulnerability. In addition to modulation of the local cervicovaginal environment, FGS has also been associated with a higher frequency of systemic CD4 T-cells expressing the chemokine receptor CCR5 (6). Tissue-entrapped eggs are also associated with clinically visible FGS-associated manifestations in the cervicovaginal mucosa (7). FGS lesions may breach the intact cervicovaginal immune barrier and are hypothesized to provide an entry point for HIV-1 infection (2, 3). However, the underlying mechanism for potential HIV-1 vulnerability in FGS has not been fully characterized and requires further investigation.

The presence of *S. haematobium* eggs in human tissue commonly provokes an inflammatory response (5). Cervicovaginal inflammation has emerged as an important risk factor for HIV-1 acquisition, with the presence of increased chemotactic cytokine concentrations, specifically macrophage inflammatory protein-1α (MIP-1α [CCL-3]), MIP-1ß [CCL-4], interleukin (IL)-8 [CXCL-8], and interferon-γ inducible protein-10 (IP-10 [CXCL-10]), conferring increased risk (8). Broadly, while pro-inflammatory cytokines are central in recruiting and activating HIV-1 target cells, they also propagate a cascade of downstream cellular processes that enact functions central to HIV-1 pathogenesis (9). The presence of pro-inflammatory cytokines in the female genital tract may also be associated with HIV-1 replication (via stimulation of transcription factors) (10), an increased frequency and activation of HIV-1 target cells (9, 11, 12) and proteomic signatures suggestive of tissue remodeling that may compromise cervicovaginal barrier function (12).

Sexually transmitted infection (STI) and a "non-optimal" cervicovaginal microbiota contribute to a vaginal pro-inflammatory environment (11, 13), and are thus important risk factors for HIV-1 acquisition (14, 15), but elevated cervicovaginal cytokine and chemokine levels have also been detected in their absence (8). In addition to STI and cervicovaginal microbiota, a number of additional biological and behavioral factors influence the levels of detectable soluble immune proteins in the female genital tract, including the presence of semen (16), cervical ectopy (16), use of hormonal contraception (17), menstrual cycle (18), and intravaginal cleansing practices (13, 17).

FGS may be an unmeasured co-factor contributing to cervicovaginal inflammatory signatures in endemic sub-Saharan African populations (8, 11). *S. haematobium* infection, in the absence of evaluation for genital involvement, has been associated with altered levels of systemic (19) and cervicovaginal cytokines (20). While male genital schistosomiasis has been associated with elevated seminal fluid cytokine concentrations (21), little is known regarding the human cervicovaginal environment in FGS or the role of the immune response. We hypothesize that FGS modulates the cervicovaginal immune environment and that evidence of FGS-associated cervicovaginal inflammation may provide insight into a causal mechanism for the association between FGS and HIV-1. In this cross-sectional study, we evaluate cervicovaginal cytokines and chemokines in women with and without FGS.

METHODS

Study Setting and Participants

The cross-sectional bilharzia and HIV (BILHIV) study was nested in HPTN 071 (PopART), a cluster randomized trial to measure the impact of an HIV-1 combination prevention package (22). HIV-1 incidence was measured in an HPTN 071 (Pop-ART) Population Cohort comprised of one randomly selected adult (18 to 44 years of age) from a random sample of households in each community who provided data and blood samples at baseline, 12, 24, and 36 months (22). After the 36-month HPTN 071 (PopART) visit, trained community workers made home visits between January and August 2018 to women who had expressed interest in the BILHIV study (23). Women

were eligible if they were 18–31 years old, not pregnant, sexually active, and resident in one of the two urban communities that participated in HPTN 071 (PopART) in Livingstone, Zambia. Following written informed consent, the BILHIV study home visit included a questionnaire, genital self-sampling (cervical and vaginal), and a urine specimen, as previously described (23).

Clinic-Based Sample Collection

Within days of self-sampling, enrolled women who were not currently menstruating were invited to attend Livingstone Central Hospital cervical cancer screening clinic, where one of two trained midwives performed a cervicovaginal lavage (CVL). Cervicovaginal images were captured with a portable colposcope (MobileODT, Tel Aviv, Israel) and were evaluated by one author (EFK) for the presence of any of the four recognized FGS cervicovaginal manifestations: homogenous yellow sandy patches, grainy sandy patches, rubbery papules, and abnormal blood vessels (24). Women having these manifestations (24) and women with any positive urine or genital *Schistosoma* diagnostic were treated free of charge with 40 mg/kg praziquantel. Testing for STI was not performed at the point-of-care and participants with suspected STI were offered syndromic management, as per local guidelines (25).

CVL Specimen Processing

After speculum insertion, normal saline (10 ml) was flushed continuously with a bulb syringe across the cervix and vaginal walls for 1 min and collected from the posterior fornices. CVL fluid was transferred to a 15 ml conical polypropylene tube and stored temporarily in a refrigerator (4°C) on ice until transfer to the laboratory. Protease inhibitor (Cocktail Set I, Calbiochem, Merck Millipore, Darmstadt, Germany) was added to one 1.5 ml aliquot for cytokine and chemokine testing and stored at −80°C, as previously described (17). Specimens were stored for a maximum of 20 months (range 12–20) and were not previously thawed. After thawing, specimens were centrifuged at 320g for 10 min and the supernatant removed. CVL color was visually assessed and a 10 µl aliquot was placed on a Hemastix test strip (Siemens, Erlangen, Germany). As per the manufacturer's instructions, CVL hemoglobin concentrations were recorded after comparing the test strip with color categories representing approximate quantities of erythrocytes (ery) per µL: none, trace, low (25 ery/µL), moderate (80 ery/µL), high (200 ery/µL) (17).

Multiplex Bead Based Assays

Luminex MAGPIX© was used to measure concentrations of seventeen soluble cytokines and chemokines using MILLIPLEX Human Cytokine/Chemokine Magnetic Bead kits (Merck Millipore, Darmstadt, Germany) according to the manufacturer's instructions and recommendations for dilute samples, i.e. CVL. The concentrations of eotaxin (CCL-11), interferon-gamma (IFN-γ), IL-10, IL-13, IL-15, IL-17A, IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8 (CXCL-8), IP-10 (CXCL-10), monocyte chemoattractant protein (MCP-1) (CCL-2), MIP-1α, (CCL-3), MIP-1β (CCL-4) and tumor necrosis factor-α (TNF-α) were measured in undiluted CVL in duplicate. The lower limit of detection was between 0.26 and 5.66 pg/ml for the 17 cytokines and chemokines measured (**S1 Table**). Using a Luminex MAGPIX© bioanalyzer and xPONENT software (version 4.2), the median fluorescent intensity was measured, background-adjusted, and converted into analyte concentrations using a 5 parameter logistic regression equation to interpolate standard curves. To minimize between-plate variations in cytokine and chemokine concentrations, two specimen controls were included in duplicate across plates and equal proportions of specimens with FGS, *probable* FGS, and FGS *negative* were distributed across six 96-well plates (8). Cytokine or chemokine concentrations below the lower limit of quantification (LLOQ) were imputed to be the midpoint of the lowest concentration for each analyte and zero and concentrations above the upper limit of quantification were imputed as the highest concentration for each analyte.

HIV-1

Laboratory-based fourth-generation HIV-1 testing (Abbott Architect HIV Ag/Ab Combo Assay). was performed for HPTN 071 (PopART) Population Cohort participants at each study visit (22).

Circulating Anodic Antigen

A lateral flow assay utilizing up-converting reporter particles for the quantification of CAA was performed on urine samples at the Leiden University Medical Center (LUMC), as previously described (23, 26). CAA levels reflect the burden of live schistosomes and decline after successful treatment with praziquantel (27, 28). Analyzing the equivalent of 417 µl urine (wet reagent, UCAA***hT***417), a CAA value of >0.6 pg/ml was considered positive (28).

PCR for Detection of *Schistosoma* DNA

DNA extraction and PCR set up was performed at LUMC, using a custom automated liquid handling station (Hamilton, Switzerland), as previously described (23). DNA was extracted from 200 µl of specimen (cervical swab, vaginal swab, CVL): with QIAamp spin columns (QIAGEN Benelux; Venlo, The Netherlands). Detection of the schistosome-specific internal-transcribed-spacer-2 (ITS2) target was performed by real-time PCR as previously described (23, 29). This PCR does not differentiate between *Schistosoma* species. DNA amplification and detection were performed with the CFX96 Real Time PCR Detection System (BioRad, California, USA). The output in cycle quantification value (Cq), reflecting the parasite-specific DNA load in the tested sample, was analyzed using BioRad CFX software. Parasite DNA loads were categorized by the following pre-specified Cq thresholds: high (Cq<30), moderate (30≤ Cq <35), low (35≤ Cq <50) and negative (no Cq detected), as previously described (30).

STI Detection

We quantified *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* using the S-DiaCTNG™ (for *C. trachomatis* and *N. gonorrhea*) and S-DiaMGTV™ (for *M. genitalium* and *T. vaginalis*) (both Diagenode Diagnostics, Seraing, Belgium) on DNA obtained from cervical swabs at Ghent University (Ghent, Belgium) according to the manufacturer's instructions. Amplification was

carried out on the LightCycler480® and the LightCyclerR 480 Software Version 1.5 (Roche, Basel, Switzerland). To quantify each of the target species, standard curves were constructed from a tenfold dilution series of DNA from *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, and T. vaginalis. Genomic DNA of *C. trachomatis* ATCC VR-571B, *T. vaginalis* ATCC 50148 and *M. genitalium* G37 was obtained from the American Type Culture Collection (ATCC). Genomic DNA from *N. gonorrhoeae* was obtained after culturing strain ATCC 43069 at 35°C ±1°C for 5 days on chocolate agar (Becton Dickinson) and extracting DNA from colonies using Roche High Pure DNA Purification kit (Roche). All DNA concentrations were determined using NanoDrop (Thermo Fisher scientific, Erembodegem, Belgium). The genomic concentrations were calculated using the described genomic sizes of the type strains. Both the standard curves and samples were run in duplicate. The number of bacteria and protozoan concentration was expressed as genome equivalents per ml (geq/ml) (31).

Ethical Considerations

The study was approved by the University of Zambia Biomedical Research Ethics Committee (reference 011-08-17), the Zambia National Health Research Authority and the London School of Hygiene and Tropical Medicine Ethics Committee (reference 14506). Permission to conduct the study was given by Livingstone District Health Office and the superintendent of Livingstone Central Hospital.

FGS Definitions

The FGS categories were defined by the results of four investigations: *Schistosoma* PCR (on DNA extracted from cervical swabs, vaginal swabs, or CVL), colposcopy image review, urine CAA, and urine microscopy. Participants were grouped by the outcomes of their diagnostic tests into three mutually exclusive categories. FGS was defined as at least one positive *Schistosoma* PCR on a genital specimen (cervical swab, vaginal swab and/or CVL). In participants with a negative *Schistosoma* PCR, *probable* FGS was defined as the presence of urinary schistosomiasis, detected with either urine CAA or urine microscopy, in combination with one of four clinical findings suggestive of FGS on any colposcope-obtained photograph (24). FGS *negative* was defined as negative results on all diagnostic assays. Participants with results for all available diagnostic tests who were *Schistosoma* genital PCR negative and did not qualify for the FGS, *probable* FGS, or FGS *negative* groups (n=190) were not eligible for study inclusion (**Figure 1**). All participants with FGS (n=28) who attended clinic follow up and provided a CVL specimen and all participants with *probable* FGS (n=25) were selected for measurement of cytokines and chemokines in CVL samples. Three FGS *negative* participants were selected for every FGS and *probable* FGS participant, using a random number generator. The FGS *negative* participants were frequency matched by age to the participants with FGS.

STATISTICAL METHODS

Participant characteristics were summarized by median and interquartile range (IQR) for continuous variables, and by frequency and percentage for categorical variables. Differences in characteristics between the FGS categories were evaluated using Fisher's exact and chi-squared tests. For cytokines or chemokines with at least 70% of sample results above the LLOQ, differences in median cytokine or chemokine

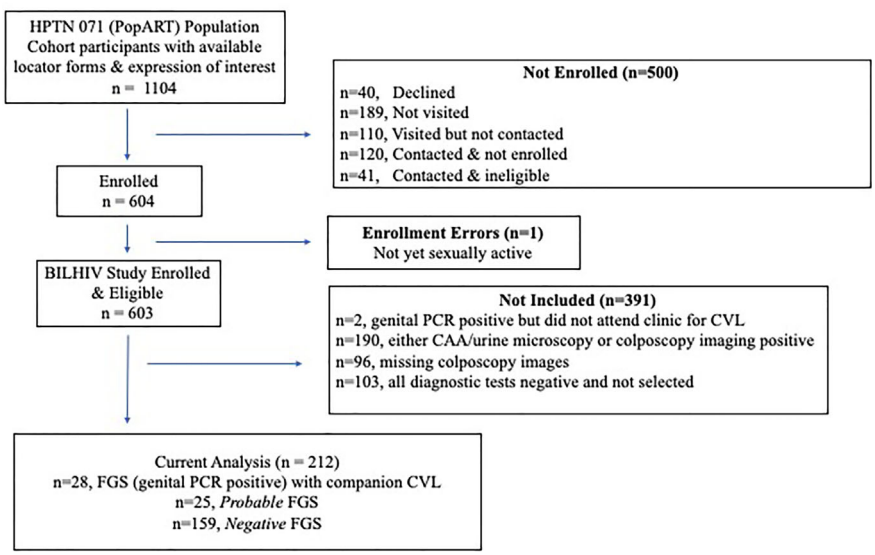


FIGURE 1 | Study Flow Diagram. Not visited (n=189)– the participant was not visited before the study closed for enrollment; Visited but not contacted (n=110)– a visit was made to the study household, but the participant could not be located (70), had relocated (39), or died (1); Contacted & not immediately enrolled (n=120)– visited but not recruited (42), out of town (18), declined to participate (60); Contacted & ineligible (n=41)– virgin (16), pregnant (17), over 31 (8).

concentrations between FGS categories were evaluated using the Wilcoxon-Mann-Whitney test. Cytokines or chemokines with <70% of the sample results above the LLOQ were analyzed as binary variables (presence/absence) and compared between FGS categories using Fisher's exact and chi-squared tests. To correct for multiple comparisons we used a Monte Carlo simulation approach with 1000 replicates (32): for each replicate the labels of the groups being compared were randomly permuted and statistical tests were repeated, to generate empirical p-values. For further analysis, cytokine or chemokine concentrations with at least 70% of sample results above the LLOQ were log-transformed to normalize their distribution, and linear regression was performed to evaluate the association between FGS and mean cytokines or chemokine in univariable and multivariable analysis and expressed with 95% confidence intervals. For the cytokines with <70% of the sample results above the LLOQ (IL-5, IL-13, IL-15, and TNF-α), logistic regression was used to evaluate the association between FGS and detectable cytokine concentrations and expressed with 95% confidence intervals. To adjust for potential confounders, we developed a causal conceptual framework (**S1 Figure**) to inform our minimal adjustment set. For the cytokines and chemokines analyzed by linear regression, we adjusted for age, education, community of residence, and the presence of any STI. Since hormonal contraception is associated with the outcome, but not the exposure (FGS), it was also included in the multivariable model to improve precision. For the cytokines analyzed by logistic regression (IL-5, IL-13, IL-15, and TNF-α), due to the relatively low number of participants with concentrations above the LLOQ, these cytokines were adjusted for age and STI. Because HIV-1 status and the presence of hemoglobin in CVL are both potentially influenced by FGS (2), and may also affect CVL cytokine or chemokine concentrations, these variables were considered to be on the causal pathway and were not included in the final multivariable model (**S1 Figure**).

Our primary hypothesis was that FGS modulates the cervicovaginal microenvironment with a secondary hypothesis that FGS may increase the concentration of selected HIV-1 acquisition associated chemokines (8). Thus, our primary analysis focused on the detection of *Schistosoma* DNA in the genital tract, comparing FGS vs FGS *negative* participants. As a secondary aim, participants with FGS and *probable* FGS were combined and compared with the FGS *negative* group.

Spearman's rank correlation was used to evaluate the strength of the relationship between individual analytes (**S2 Figure**). Since many cytokines and chemokines were correlated, we performed Principal Components Analysis (PCA) on the log-transformed analyte concentrations to generate new uncorrelated "components" that were linear combinations of the initial variables. The first two principal components captured the majority of the variability in the data and were taken forward for additional comparisons between FGS groups (**S2 Table**).

To evaluate the possible association between intensity of FGS presentation and changes in cytokine or chemokine concentrations, two *ad hoc* exploratory analyses were performed: (1) participants with ≥2 genital samples with detectable *Schistosoma* DNA levels were compared with those in the FGS *negative* group, (2) participants with a moderate/high genital *Schistosoma* DNA concentration (defined by a Cq <35 in at least one of the three examined samples) were compared with those in the FGS *negative* group.

In this study we measured the concentration of cytokines and chemokines in CVL. However, the presence of hemoglobin in CVL may serve as a surrogate marker for the presence of systemic and/or menstrual blood in the cervicovaginal environment. HIV-1 status and the presence of hemoglobin in CVL are potentially influenced by FGS (2), but may also independently affect cytokines and chemokine concentrations. Thus, we performed two sensitivity analyses, one compared participants with FGS with those in the FGS *negative* group after excluding the participants who were HIV-1 positive from both groups. A second sensitivity analysis compared participants with FGS with those in the FGS *negative* group after excluding the participants whose CVL sample displayed the presence of hemoglobin. Data were analyzed using STATA 15.1 (Stata Corporation, College Station, TX). P-values less than 0.05 were classified as demonstrating "evidence" of an association and p-values between 0.05 and 0.10 were classified as demonstrating "some evidence" of an association.

RESULTS

A total of 603 eligible women were enrolled and 212 (35.2%) were included in this study (**Figure 1**). Overall, 13.2% (28/212) of women had FGS, defined by a positive genital *Schistosoma* PCR from any of the following sites: 8.5% (18/212) cervical swab, 6.6% (14/212) vaginal swab, and 6.6% (14/212) CVL. *Probable* FGS was detected in 25 women, and 61.1% (159/262) of the women who were negative on all diagnostic tests were randomly selected for inclusion in this study.

Baseline Characteristics

The majority of the participants had received at least secondary education, were using hormonal contraception, and had detectable hemoglobin in their CVL. At the conclusion of HPTN 071 (PopART), HIV-1 prevalence was 17.0% (36/212) among the women included in this study and one-third of the women had at least one STI (**Table 1**). Active schistosome infection, defined as either a positive urine microscopy (11.8%, 25/212) or detectable CAA (20.2%, 43/212), was reported in 21.2% (45/212). A small proportion of women reported current water contact, but more than half reported childhood water contact.

There was strong evidence of a difference in community of residence between FGS, *probable* FGS, and FGS *negative* participants (p=0.001) with participants with FGS and *probable* FGS more likely to live in Community A than participants in the FGS *negative* group (**Table 1**). There were differences between the three categories of FGS status for age (p<0.001), educational

TABLE 1 | Baseline characteristics of the 212 study participants by female genital schistosomiasis (FGS) status.

Socio-behavioral Characteristics		FGS* % (n = 28)	FGS Probable* % (n = 25)	FGS Negative* % (n = 159)	p-value
Age in years	Median (IQR)	22 (20–24)	27 (23–31)	23 (22–24)	0.001
Marital Status	Single	42.9 (12)	16.0 (4)	45.9 (73)	0.04[∫]
	Married or Cohabitating	57.1 (16)	80.0 (20)	50.9 (81)	
	Divorced or Separated	0.0 (0)	4.0 (1)	3.1 (5)	
Education (highest level)	None/Any Primary School	32.1 (9)	48.0 (12)	22.0 (35)	0.04[∫]
	Any Secondary School	67.9 (19)	52.0 (13)	70.4 (112)	
	Training in a Trade	0.0 (0)	0.0 (0)	7.6 (12)	
Employment Status	Working	14.3 (4)	44.0 (11)	25.8 (41)	0.05
	Not Working	85.7 (24)	56.0 (14)	74.2 (118)	
Current Water Contact	None	100.0 (28)	84.0 (21)	86.8 (138)	0.1[∫]
	Any	0.0 (0)	16.0 (4)	13.2 (21)	
Childhood Water Contact	None	14.3 (4)	24.0 (6)	32.1 (51)	0.1
	Any	85.7 (24)	76.0 (19)	67.9 (108)	
Community of Residence	Community A	75.0 (21)	80.0 (20)	41.5 (66)	<0.001
	Community B	25.0 (7)	20.0 (5)	58.5 (93)	
Sexual behavior characteristics and STI					
Ever pregnant	No[#]	7.1 (2)	4.0 (1)	17.0 (27)	0.3[∫]
	Yes	92.9 (26)	96.0 (24)	82.4 (131)	
Age at sexual debut	Median (IQR)	16 (15–18)	17 (15–18)	17 (16–18)	0.09
Lifetime sexual partners	Median (IQR)	3 (2–4.5)	2 (1–3)	2 (1–4)	0.3
Currently Sexually Active	No[##]	10.7 (3)	8.0 (2)	16.5 (26)	0.6[∫]
	Yes	89.3 (25)	92.0 (23)	83.5 (132)	
Contraceptive Method	Implant	7.1 (2)	8.0 (2)	8.8 (14)	1.0[∫]
	Injectable	53.6 (15)	48.0 (12)	47.8 (76)	0.9
	Oral Contraceptive Pill	3.6 (1)	12.0 (3)	6.3 (10)	0.5[∫]
	Condoms	10.7 (3)	20.0 (5)	16.4 (26)	0.7[∫]
HIV-1	Not Detected	82.1 (23)	80.0 (20)	83.7 (133)	0.9
	Detected	17.9 (5)	20.0 (5)	16.4 (26)	
Chlamydia trachomatis	Not Detected	89.3 (25)	96.0 (24)	91.8 (146)	0.7[∫]
	Detected	10.7 (3)	4.0 (1)	8.2 (13)	
Neisseria gonorrhea	Not Detected	100.0 (28)	96.0 (24)	92.4 (147)	0.4[∫]
	Detected	0.0 (0)	4.0 (1)	7.6 (12)	
Mycoplasma genitalium	Not Detected	100.0 (28)	96.0 (24)	95.6 (152)	0.5[∫]
	Detected	0.0 (0)	4.0 (1)	4.4 (7)	
Trichomonas vaginalis	Not Detected	67.9 (19)	68.0 (17)	78.6 (125)	0.3
	Detected	32.1 (9)	32.0 (8)	21.4 (34)	
Any STI	Not Detected	64.3 (18)	56.0 (14)	67.3 (107)	0.5
	Detected	35.7 (10)	44.0 (11)	32.7 (52)	
Clinical Findings					
Hemoglobin in CVL (ery/μL)[ϑ]	None	28.6 (8)	25.0 (6)	36.1 (57)	0.3[∫]
	Trace	17.9 (5)	12.5 (3)	15.2 (24)	
	25	32.1 (9)	16.7 (4)	17.7 (28)	
	80	3.6 (1)	16.7 (4)	17.1 (27)	
	200	17.9 (5)	29.2 (7)	13.9 (22)	
Colposcopy Findings[ϑϑ]	Sandy Patches	22.2 (6)	76.0 (19)	0.0 (0)	N/A
	Rubbery Papule	0.0 (0)	0.0 (0)	0.0 (0)	
	Abnormal Blood Vessels	22.2 (6)	24.0 (6)	0.0 (0)	
	No FGS findings	56.0 (15)	0.0 (0)	100.0 (159)	

**FGS – Schistosoma PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; probable FGS– Schistosoma PCR negative and either positive circulating anodic antigen (CAA) or urine microscopy and suggestive expert-reviewed colposcopy imaging; FGS negative – negative Schistosoma PCR and negative CAA and negative urine microscopy and negative expert-reviewed colposcopy imaging.*

[∫]Fisher's exact test.

[#]Self-reported history of ever having a pregnancy, participants reporting "no answer" (n=1, FGS negative group) are not shown.

[##]Participants reporting "no answer" (n=1, FGS negative group) are not shown.

[ϑ]Hemoglobin was measured with Hemastix® test strips using a color chart measured in erythrocytes (ery) per μl.

[ϑϑ]Colposcopy findings were included in the study inclusion criteria, so no p-value is shown; one participant with FGS did not have interpretable colposcopy results. Images were interpreted based on the presence of sandy patches or rubbery papules, if these findings were present, the additional finding of abnormal blood vessels was not noted.

attainment (p=0.04), employment (p=0.05), and marital status (p=0.04) with participants in the *probable* FGS group more likely to be older, have a primary school education, be employed, and be married than FGS and FGS *negative* participants. Other characteristics were similar by FGS status.

Expression Profiles of Cytokines and Chemokines in CVL

The mean, median and range of concentrations (pg/ml) of the 17 cytokines and chemokines measured are displayed in **S1 Table**. The distributions of log-transformed concentrations (median

and interquartile range) of the 17 cytokines and chemokines are displayed by FGS status in **Figure 2**.

FGS Signature - Crude and Adjusted Expression Profiles

Compared to FGS *negative* women, IL-5 was elevated in participants with FGS (**Table 2**, **Figure 2**, crude p-value 0.02, p-value after adjustment for multiple testing 0.14). Compared to FGS *negative* women, women with FGS had similar expression profiles of chemokines that predicted HIV-1 acquisition risk in a South African study (MIP-1α [CCL-3], MIP-1β [CCL-4], IL-8 [CXCL-8], and IP-10 [CXCL-10]) (8). This was confirmed after adjusting for age, STI, community of residence, education, and use of hormonal contraception (**Figure 3**). Principal Components Analysis identified that two Principal Components accounted for 60.0% of the variability in the data (**S2 Table**). Taken forward, there was no difference in mean scores for these two Principal Components by FGS status (**S3 Figure**).

When the FGS and *probable* FGS groups were combined, in the crude analysis (**S4 Figure**) and after adjustment for possible confounders, participants with FGS/*probable* FGS had higher concentrations of TNF-α than FGS *negative* participants (**S5 Figure**, p=0.03, p-value adjusted for multiple testing 0.09).

Exploratory Analyses – Clinical Disease Burden

In an exploratory analysis of participants (n=15) with a higher FGS burden defined as ≥2 *Schistosoma* PCR positive genital specimens, there was evidence of an elevated concentration of cytokines IL-4, IL-5, IL-13, IL-15 compared to FGS *negative* women (**Figure 4**). This association remained after adjusting for potential confounders (**Figure 5**). After adjustment for multiple comparisons strong evidence remained that IL-4 (multiple testing adjusted p=0.037) and IL-5 (multiple testing adjusted p<0.001) were associated with FGS.

Exploratory Analyses – *Schistosoma* DNA Concentration

In a further exploratory analysis of women (n=15) with FGS and moderate or high genital *Schistosoma* DNA concentration (*Schistosoma* PCR Cq<35) there was evidence that the concentrations of cytokines IL-1α, IL-4, IL-5, IL-13, IL-15, and TNF-α were elevated in participants with *Schistosoma* PCR Cq<35 compared to FGS *negative* women (**Figure 6**). After adjustment for potential confounders, evidence remained that IL-4, IL-5, IL-15, and TNF-α were elevated in participants with moderate/high *Schistosoma* DNA concentration compared to FGS *negative* women (**Figure 7**). After adjustment for multiple comparisons, strong evidence remained that the associations for IL-5 (multiple testing adjusted p=0.001) and TNF-α (multiple testing adjusted p=0.045) were unlikely to have occurred by chance. When comparing the participants with moderate/high *Schistosoma* DNA concentration and the participants with ≥2 PCR positive genital specimens, 11 women overlapped between groups.

Sensitivity Analyses – HIV-1 and CVL Hemoglobin

We performed sensitivity analyses removing participants with HIV-1 infection or the presence of hemoglobin in CVL from the comparison between FGS and *negative* FGS groups. When n=31 participants with HIV-1 from the FGS and FGS *negative* groups were excluded from the analysis, there was no difference in the expression profiles of cytokines or chemokines in women with FGS compared to FGS *negative* women (**S6 Figure**). When n=139 participants with any detectable hemoglobin in CVL were removed from the analysis (**S7 Figure**), there was some evidence that concentrations of IL-17A and IL-8 (CXCL-8) were higher in participants with FGS (n=8) compared to the FGS *negative* group (n=57) (p=0.13 after allowing for multiple testing).

DISCUSSION

This study is the first to describe expression patterns of cytokines and chemokines in human FGS, diagnosed by *Schistosoma* PCR from vaginal swabs, cervical swabs, and CVL. The immune environment in helminth infection is often characterized as T helper 2 (Th2) biased and involves the orchestration of cytokines (IL-4, IL-5, IL-13), antibodies, and regulatory cells (33). We did not detect an association between FGS, defined as *Schistosoma* DNA detected in genital PCR specimens, and a change in

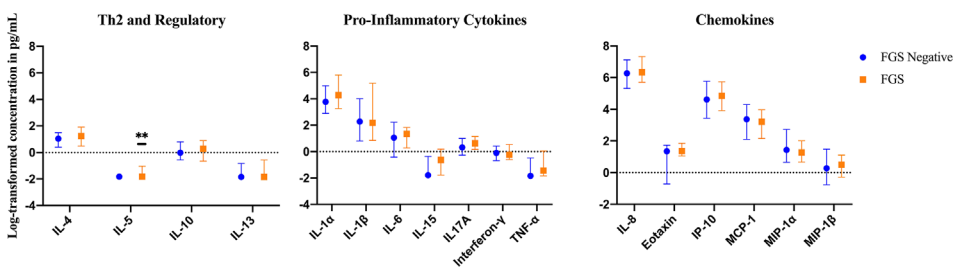


FIGURE 2 | Median with interquartile range of the log-transformed crude concentrations of eleven cytokines and six chemokines in cervicovaginal lavage of participants with (FGS: n=28) and without female genital schistosomiasis (FGS negative: n=159) [χ]. FGS – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative genital PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging. [χ]p-value after adjustment for multiple testing with a Monte-Carlo simulation approach, p=0.14 p-value symbol legend: **p < 0.05.

TABLE 2 | Crude and adjusted associations (with 95% confidence intervals) of FGS* status with concentrations of cytokines and chemokines in cervicovaginal lavage**.

Analyte	%(n) above LLOQ	FGS Negative ‡n=159	FGS n=28	p-value‡‡	GMR FGS vs FGS Negative (n=28) †	p-value † †
Linear Regression - *Analytes with >70% above LLOQ*						
Eotaxin	73.6 (156)	3.85	3.92	0.22	1.48 (0.95–2.32)	0.08
Interferon–γ	84.0 (178)	0.91	0.80	0.71	0.98 (0.64–1.51)	0.94
IL–10	90.1 (191)	0.98	1.33	0.75	1.02 (0.65–1.61)	0.93
IL–17A	93.4 (198)	1.38	1.86	0.14	1.37 (0.85–2.23)	0.64
IL1–α	99.5 (211)	44.00	73.08	0.17	1.24 (0.63–2.44)	0.19
IL1–β	93.4 (198)	9.79	9.05	0.38	1.59 (0.56–4.51)	0.52
IL–4	96.2 (204)	2.79	3.42	0.20	1.41 (0.94–2.12)	0.09
IL–6	87.7 (186)	2.85	3.81	0.67	1.14 (0.56–2.35)	0.71
IL–8	100.0 (212)	526.03	566.68	0.33	1.29 (0.75–2.19)	0.34
IP–10	100.0 (212)	101.86	128.38	0.42	1.34 (0.68–2.66)	0.39
MCP–1	100.0 (212)	29.04	24.68	0.79	0.98 (0.53–1.83)	0.95
MIP1–α	98.1 (208)	4.23	3.61	0.15	0.62 (0.36–1.08)	0.09
MIP1–β	84.0 (177)	1.32	1.64	0.67	1.05 (0.54–2.05)	0.89
Logistic Regression - *Analytes with <70% above LLOQ*						
Analyte	**%(n) above LLOQ**	**FGS Negative %(n=159)**	**FGS %(n=28)**	**p-value$^{\chi}$**	**OR FGS vs FGS Negative$^{\chi\chi}$**	**p-value#**
IL–5	17.0 (36)	13.84 (22)	32.1 (9)	0.02	3.44 (1.30–9.05)	0.02
IL–13	32.6 (69)	32.7 (52)	35.7 (10)	0.76	1.17 (0.50–2.75)	0.72
IL–15	47.2 (100)	44.7 (71)	60.7 (17)	0.12	1.91 (0.84–4.36)	0.12
TNF–α	41.0 (87)	35.9 (57)	50.0 (14)	0.15	1.85 (0.81–4.22)	0.14

FGS, female genital schistosomiasis; GMR, geometric mean ratio; LLOQ, lower limit of quantification.

**FGS – Schistosoma PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS negative – negative Schistosoma PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging.*

***Concentrations are reported in pg/ml.*

‡n=25 Participants with probable FGS are not shown.

‡‡Rank sum p-value.

†Adjusted for age, STI, educational level attained, community of residence, and hormonal contraception.

† †F-test p-value.

χChi-squared p-value.

χχAdjusted for age and STI.

#Likelihood ratio test p-value.

expression pattern of cytokines or chemokines in CVL including those associated with HIV-1 acquisition in a South African study (8). Compared to the FGS *negative* participants, the Th2 cytokine IL-5 was elevated in the participants with FGS, however after adjustment for multiple comparisons we cannot exclude that this finding may be due to chance.

Previous work on male genital schistosomiasis has shown that infection intensity, defined by seminal egg count, is strongly associated with elevated seminal cytokine concentrations including Th2 (IL-4), regulatory (IL-10), Th1 (IFN-γ) and pro-inflammatory (TNF-α) cytokines (21). Thus, we evaluated FGS burden by performing two exploratory analyses, the results of which show a Th2 expression pattern. First, we investigated the association between multiple PCR-positive genital specimens as a potential proxy marker of higher FGS burden in 15 women with ≥ two positive genital specimens for *Schistosoma* DNA. We also investigated whether *Schistosoma* DNA concentrations in genital samples might be associated with a change in cytokine concentrations in 15 participants with FGS and moderate/high genital *Schistosoma* DNA concentrations (defined as *Schistosoma* PCR Cq<35). After adjusting for potential confounders, the exploratory analyses found a higher cervicovaginal Th2 cytokine response (IL-4, IL5-, IL-13) in participants with ≥ two positive specimens and participants with higher genital *Schistosoma* DNA concentrations. This is not unexpected considering that a Th2 biased immune response is associated with helminth infection (33) and *S. haematobium* exposure in both human and murine hosts (34, 35). There was evidence for higher concentrations of IL-5 after adjustment for multiple comparisons across both exploratory analyses. IL-5 induces eosinophil maturation and an IL-5 response to *Schistosoma* antigens has been associated with microhematuria in children with *S. haematobium* infection (36). There was evidence for higher concentrations of IL-4 after adjustment for multiple comparisons in the exploratory analyses of multiple PCR-positive genital specimens for *Schistosoma* DNA. In the Th2 response, IL-4 directly regulates T-cell differentiation and proliferation (37). The finding of elevated levels of pro-inflammatory TNF-α in the exploratory analyses of participants with moderate/high *Schistosoma* DNA concentrations after adjustment for potential confounders and multiple comparisons, suggests that FGS may promote a mixed Th2 and pro-inflammatory response. TNF-α may be associated with *Schistosoma* granuloma formation (38) and in children with *S. haematobium* infection, TNF-α production has been associated with ultrasound-determined urinary bladder morbidity (39).

We found IL-13 and IL-15 to be associated with a higher clinical FGS burden and higher genital *Schistosoma* DNA concentrations after adjusting for potential confounders, although these associations no longer remained after adjustment for multiple comparisons. Predominantly produced by macrophages, IL-15 is

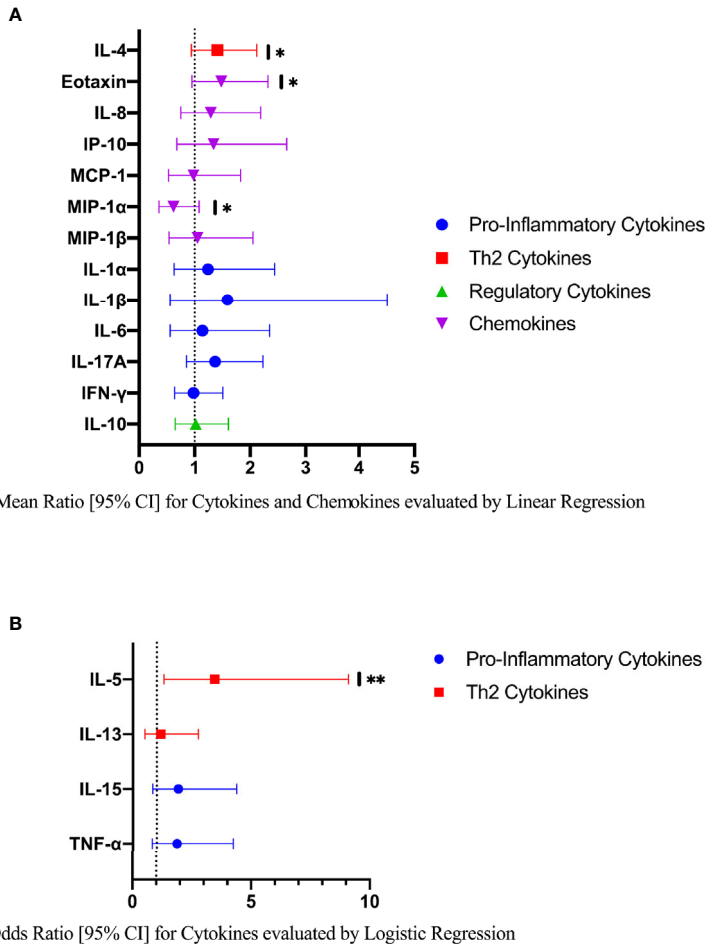


FIGURE 3 | Comparison of the concentration or presence of eleven cytokines and six chemokines in cervicovaginal lavage of participants with (FGS, n=28) and without (FGS negative, n=159) female genital schistosomiasis[χ]. **(A)** Concentrations of eotaxin, IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, MCP-1, MIP-1α, and MIP-1β were compared between FGS and FGS negative participants by linear regression and adjusted for age, community of residence, education, presence of sexually transmitted infection and hormonal contraceptive use, with results shown as geometric mean ratios with 95% CI. **(B)** Presence/absence of IL-5, IL-13, IL-15 and TNF-α were compared by logistic regression and adjusted for age and sexually transmitted infection, with results shown as odds ratio with 95% CI. The line at 1 indicates the value at which there is no difference between the FGS and FGS *negative* groups. p-value symbol legend: *p < 0.1 **p < 0.05. [χ]female genital schistosomiasis – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative genital PCR and negative urine circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging.

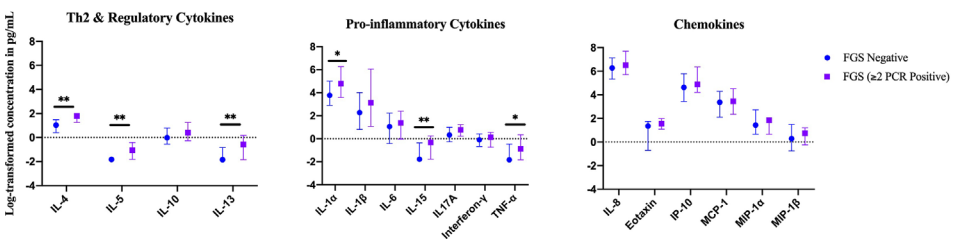


FIGURE 4 | Median with interquartile range of the log-transformed crude concentrations of eleven cytokines and six chemokines in cervicovaginal lavage by FGS[χ] burden (*Schistosoma* PCR in ≥ two genital specimens: n=15, FGS negative: n=159) [χχ]. [χ]FGS – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative *Schistosoma* PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging. [χχ]p-value after adjustment for multiple testing with a Monte-Carlo simulation approach, p < 0.001. p-value legend *p < 0.1 **p < 0.05.

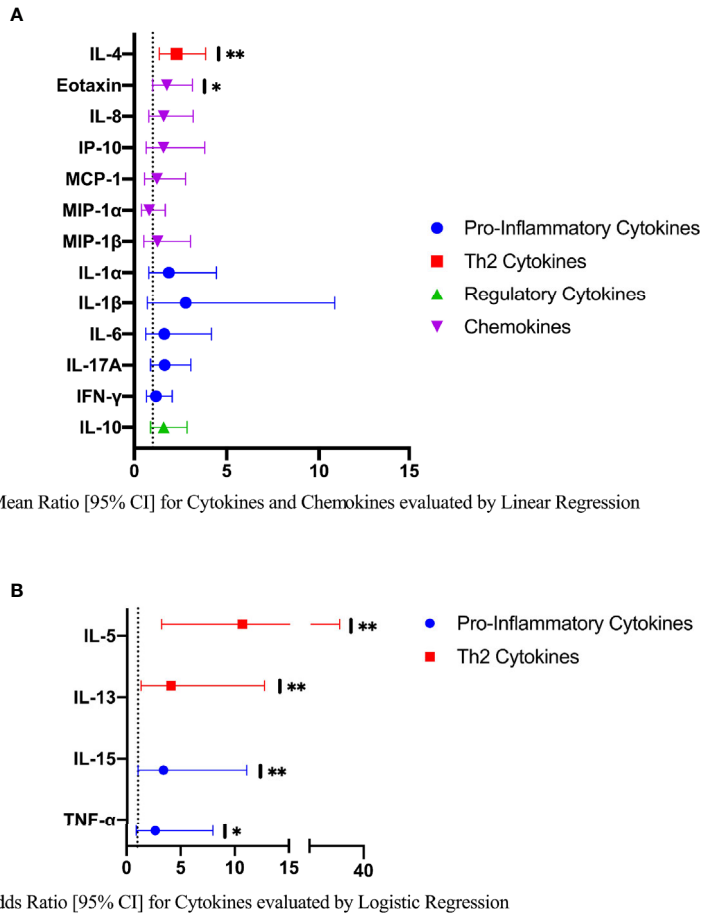


FIGURE 5 | Comparison of the concentrations or presence of eleven cytokines and six chemokines in cervicovaginal lavage in participants with greater FGS burden (*Schistosoma* PCR positive in ≥ two genital specimens, n=15) and participants without female genital schistosomiasis (n=159) [χ]. **(A)** Concentrations of eotaxin, IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, MCP-1, MIP-1α, and MIP-1β were compared between FGS and FGS *negative* participants by linear regression adjusted for age, community of residence, education, presence of sexually transmitted infection and hormonal contraceptive use, with results shown as geometric mean ratios with 95% CI. **(B)** Presence/absence of IL-5, IL-13, IL-15 and TNF-α were compared by logistic regression and adjusted for age and sexually transmitted infection, with results shown as odds ratio with 95% CI. The line at 1 indicates the value at which there is no difference between the FGS and FGS *negative* groups. p-value symbol legend: *p <0.1 **p <0.05. [χ]female genital schistosomiasis – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative *Schistosoma* PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging.

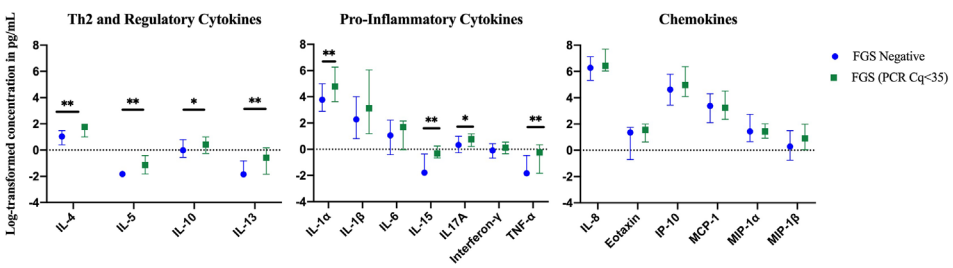


FIGURE 6 | Median with interquartile range of the log-transformed crude concentrations of eleven cytokines and six chemokines in cervicovaginal lavage by FGS burden (*Schistosoma* DNA concentration Cq <35 in any genital specimen, n=15, FGS *negative*:n=159) [χ]. FGS – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative *Schistosoma* PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging. [χ]p-value after adjustment for multiple testing with a Monte-Carlo simulation approach, p=0.001 p-value symbol legend: *p < 0.1 **p < 0.05.

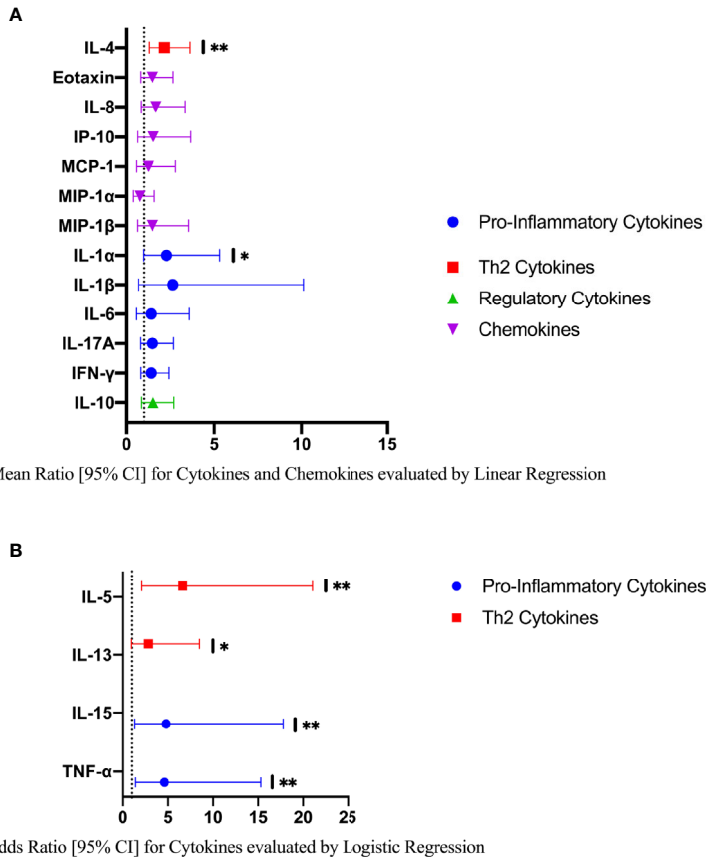


FIGURE 7 | Comparison of the concentration or presence of eleven cytokines and six chemokines in cervicovaginal lavage in participants with moderate to high *Schistosoma* DNA concentrations (Cq <35 in any genital specimen, n=15) and participants without female genital schistosomiasis (n=159) [χ]. **(A)** Concentrations of eotaxin, IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, MCP-1, MIP-1α, and MIP-1β were compared by linear regression adjusted for age, community of residence, education, presence of sexually transmitted infection and hormonal contraceptive use. Results are represented by geometric mean ratios with 95% CI. **(B)** Presence/absence of IL-5, IL-13, IL-15 and TNF-α were compared by logistic regression and adjusted for age and sexually transmitted infection. Results are indicated by odds ratio with 95% CI. The line at 1 indicates the value at which there is no difference between the FGS and FGS *negative* groups. p-value symbol legend: * <0.1, ** <0.05. [χ]Female genital schistosomiasis – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative *Schistosoma* PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging.

a pro-inflammatory, immunomodulatory cytokine that stimulates T, B, and Natural Killer cells (40). Lower levels of IL-15 have been reported in the cervicovaginal environment of women with *S. haematobium* infection, with between-study differences potentially related to genital *Schistosoma* DNA detection (20). The Th2 cytokine IL-13 is thought to be an important driver of collagen deposition in the *Schistosoma* egg granuloma (41), ultimately leading to fibrosis. Indeed, IL-13 expression levels have been associated with the severity of hepatic fibrosis in *S. mansoni* infection (42). The exploratory sub-group analyses thus support previous work describing that *S. haematobium* may modulate the genital immune environment (20, 21). As a protracted Th2 response is often associated with schistosomiasis-related morbidity (43), our study findings warrant further investigation. The current study included many statistical tests, and these were allowed for using a permutation testing approach. However, we cannot exclude the possibility that some of the associations are due to chance. Thus, it may be more instructive to consider patterns in cytokine signatures (Th2, pro-inflammatory) across analyses, rather than interpreting significance testing for any one cytokine in isolation. Although strong evidence remained for some of the associations found in the exploratory analyses of FGS burden after adjusting for potential confounders and multiple comparisons, as exploratory analyses, these findings should be viewed as hypothesis generating.

Many hypotheses have been put forward regarding the mechanism of HIV-1 vulnerability in women with FGS, with evidence of both local mucosal factors and systemic immunomodulation (2, 4–6). In a South African study, women who acquired HIV-1 in the CAPRISA tenofovir gel trial had higher concentrations of the chemotactic cytokines MIP1-α (CCL-3), MIP1-β (CCL-4), IL-8 (CXCL-8), and IP-10 (CXCL-10) prior to seroconversion than women who did not seroconvert (8). In the current study, the concentrations of the aforementioned chemotactic chemokines were not higher in women with FGS compared to the FGS *negative* group. While not yet studied in *S. haematobium*, herpes simplex virus type 2 infection stimulates

TNF-α production in dendritic cells, enhancing the expression of the chemokine co-receptor CCR5 and stimulating HIV-1 replication (44). Further research is needed to elucidate mechanisms for the association of FGS with HIV-1 vulnerability, and these may include schistosome-related impact on mucosal and systemic immunity, including the activation of CD4 trafficking to the genital mucosa (45), or modification in systemic (46) or cervical (20) gene expression, specifically related to the regulation of transcription, the inflammatory response or tissue fibrosis.

Cervical tissue containing *S. haematobium* eggs is more vascular (4) compared to non-egg containing tissue. Clinically, these abnormal blood vessels can be found encircling sandy patches and contact bleeding has been associated with FGS (47). Thus, FGS studies using cervicovaginal lavage are likely to be burdened by the presence of hemoglobin (48). Since HIV-1 and the presence of hemoglobin in CVL are potentially on the causal pathway between FGS and a change in cytokine or chemokine concentrations, we were unable to adjust for these possible confounding variables in a multivariable model. A sensitivity analysis removing participants with HIV-1 showed no change in the association between FGS and cytokine or chemokine concentrations. HIV-1 infection modulates the cervicovaginal immune environment in women with detectable cervicovaginal HIV-1 RNA (49) and we did not have complete data on plasma viral load in this cohort. Additionally, the analysis to remove participants with any CVL hemoglobin detection was limited by loss of power. Once participants who had any CVL hemoglobin were removed from the analysis, there was evidence of higher concentrations of IL-17A and IL-8 (CXCL-8) in participants with FGS (n=8) compared with FGS *negative* participants (n=57). This finding, however, was less robust after adjusting for multiple comparisons. Considering the potential loss of the FGS phenotype when excluding women with CVL hemoglobin and the small and likely non-representative sample size, these findings should be interpreted with caution.

Our study has a number of strengths. We are the first to describe the cervicovaginal immune environment in women with *Schistosoma* PCR-defined FGS and this study illustrates the importance of evaluating FGS burden. Defining FGS based on the detection of *Schistosoma* DNA in the female genital tract by PCR results provides a higher certainty of genital involvement and a quantitative reference standard compared to visually-diagnosed FGS or the use of urine diagnostics alone. Due to the small numbers of FGS cases, we employed a matching strategy wherein participants with FGS were frequency matched with FGS *negative* participants. To reduce the risk of selection bias, we used a random number generator to randomly select controls and matched on age group. Multiplex bead-based assays have a precedent for use in CVL (8, 11, 14, 17) and we examined a variety of soluble immune proteins including chemokines, Th1, Th2, pro-inflammatory and regulatory cytokines for a broad overview of the cervicovaginal immune environment. Another strength is that we present both crude and adjusted outcomes to facilitate comparisons in future study settings.

While our study has multiple strengths, there are also some relevant limitations. The study was conducted in a low-prevalence area and the number of FGS cases in the main and exploratory analyses is small. We selected a sub-sample of the cohort for multiplex bead-based immunoassays. This may limit the generalizability of the proportions presented for demographic variables or FGS and schistosomiasis prevalence. Secondly, we were unable to measure a number of behavioral and biological factors that also affect cervicovaginal soluble immune protein expression patterns including the presence of bacterial vaginosis (13), HSV-2 status (11), intra-vaginal cleansing practices (17), vaginal pH, menstrual cycle phase (18), body weight (50) and recent sexual contact (16). It is also undetermined how these same factors may influence *Schistosoma* DNA concentrations. Thus, we cannot exclude unmeasured and residual confounding. Additionally, due to the cross-sectional study design, we were also unable to assess the long-term impact of the cytokine expression profiles or to determine the duration of FGS infection. Though the cytokines and chemokines we measured are well-known biomarkers for inflammation and disease, it is a limitation that we did not have companion flow cytometry, biopsy, or transcriptomic data for a more detailed evaluation of cellular and histological processes. Additionally, CVL is dilute and the concentrations of the cytokines and chemokines we measured were small. This could potentially be ameliorated in future studies with the use of a menstrual cup to collect genital fluid (51).

FGS is thought to be a chronic infection of the female genital tissue, with initial infection and the development of genital lesions occurring during childhood water contact (52) that persist into adulthood, often even despite treatment with praziquantel (53). The chronicity of FGS lesions may have an impact on the cervicovaginal immune environment and a longitudinal study is needed to document the FGS immune environment in a spectrum of FGS stages before interventions can be based on our exploratory observations. Further work investigating the cervicovaginal immune environment in FGS may impact diagnostic, preventative, and therapeutic options as well as potentially providing additional information on HIV-1 vulnerability.

In conclusion, this study does not show a difference in the cervicovaginal immune environment by *Schistosoma* PCR-defined FGS status. However, two exploratory analyses suggest that there may be a relationship between higher genital *Schistosoma* DNA concentrations or multiple PCR positive genital specimens and a Th2 and pro-inflammatory modulation of the cervicovaginal immune environment, as measured by elevated cytokine concentrations. FGS may alter the female genital tract immune environment, but a larger longitudinal study in a high FGS prevalence area is needed to better define the role of FGS in HIV-1 acquisition.

DATA AVAILABILITY STATEMENT

Anonymised data from the HPTN 071 (PopART) study that support the findings of this study can be made available by the HPTN 071 (PopART) study team, subject to an application process. Further details can be obtained from AB (amaya.bustinduy@lshtm.ac.uk).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Zambia Biomedical Research Ethics Committee (reference 011-08-17), the Zambia National Health Research Authority and the London School of Hygiene and Tropical Medicine Ethics Committee (reference 14506). Permission to conduct the study was given by Livingstone District Health Office and the superintendent of Livingstone Central Hospital. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AS – conceptualization, data curation, formal analysis, investigation, BILHIV project administration, visualization, writing - original draft preparation. EW – conceptualization, data curation, formal analysis, supervision, visualization, writing - original draft preparation. CP– investigation, writing – review and editing. CRP – BILHIV project administration, writing – review and editing. TM – investigation, writing – review and editing. EK – investigation, writing – review and editing. MM – investigation, writing – review and editing. JM – investigation, writing – review and editing. MMM – conceptualization, resources, writing – review and editing. JC – conceptualization and resources. GD – investigation, writing – review and editing. PC – investigation, writing – review and editing. HA – resources, writing – review and editing. RH – resources, supervision, writing – review and editing. IH – resources, supervision, writing – review and editing. GM – resources, writing – review and editing. PC – investigation, writing – review and editing. LL – investigation, writing - review and editing. HH – conceptualization, supervision, writing – review and editing. SF – conceptualization, supervision, writing original draft preparation. AB – conceptualization, funding acquisition, supervision, visualization, writing - original draft preparation. All authors contributed to the article and approved the submitted version.

FUNDING

AB received funding from the Wellcome Trust (Award 205954/Z/17/Z) and the Dowager Countess Eleanor Peel Trust. EW and RH received funding from MRC Grant Reference MR/K012126/1, and SF received salary from MRC Grant Reference MR/N023692/1. These awards are jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 program supported by the European Union. HPTN 071 (PopART) was supported by the National Institute of Allergy and Infectious Diseases (NIAID) under Cooperative Agreements UM1-AI068619, UM1-AI068617, and UM1-AI068613, with funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR); the International Initiative for Impact Evaluation with support from the Bill and Melinda Gates Foundation; the NIAID, the National Institute on Drug Abuse, and the National Institute of Mental Health, all part of the National Institutes of Health. EK was supported by South-Eastern Regional Health Authority, Norway project #2016055.

ACKNOWLEDGMENTS

We would like to thank the study participants for their contribution to this work. We recognize the BILHIV Study team, supervisor Namakau Chola, and field workers Ethel Mwansa, Mwiingana Lukonga, Ruth Mwanza, Mervis Kantukaleza, and Judith Lungu for their hard work, enthusiasm, and commitment. We also acknowledge tremendous support in Livingstone from Clement Mwakamui (Zambart). We recognize Kevin Tetteh (LSHTM) for generously providing resources and support to run the multiplex bead-based assays. We gratefully acknowledge Eric AT Brienen (LUMC) for performing the *Schistosoma* PCR analysis and Claudia J. de Dood (LUMC) and Pytsje T. Hoekstra (LUMC) for performing the CAA analysis. We would like to thank Jo-Ann Passmore (University of Cape Town), Lindi Masson (Burnett Institute), Michael Hsieh (Children's National), and Hazel Dockrell (LSHTM) for instructive consultations regarding analyte selection. We are grateful to Michael Hsieh (Children's National) for his critical review of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.620657/full#supplementary-material

REFERENCES

1. UNAIDS. *UNAIDS Global AIDS Update, Communities at the Centre 2019* . Available at: https://www.unaids.org/en/20190716_GR2019_communities (Accessed November 2, 2020).
2. Sturt AS, Webb EL, Francis SC, Hayes RJ, Bustinduy AL. Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition. *Acta Trop* (2020) 209:105524. doi: 10.1016/j.actatropica.2020.105524
3. Kjetland EF, Ndhlovu PD, Gomo E, Mduluza T, Midzi N, Gwanzura L, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. *AIDS* (2006) 20(4):593–600. doi: 10.1097/01.aids.0000210614.45212.0a
4. Jourdan PM, Roald B, Poggensee G, Gundersen SG, Kjetland EF. Increased vascularity in cervicovaginal mucosa with Schistosoma haematobium infection. *PloS Negl Trop Dis* (2011) 5(6):e1170. doi: 10.1371/journal.pntd.0001170
5. Jourdan PM, Holmen SD, Gundersen SG, Roald B, Kjetland EF. HIV target cells in Schistosoma haematobium-infected female genital mucosa. *Am J Trop Med Hyg* (2011) 85(6):1060–4. doi: 10.4269/ajtmh.2011.11-0135
6. Kleppa E, Ramsuran V, Zulu S, Karlsen GH, Bere A, Passmore JA, et al. Effect of female genital schistosomiasis and anti-schistosomal treatment on monocytes, CD4+ T-cells and CCR5 expression in the female genital tract. *PLoS One* (2014) 9(6):e98593. doi: 10.1371/journal.pone.0098593
7. Kjetland EF, Poggensee G, Helling-Giese G, Richter J, Sjaastad A, Chitsulo L, et al. Female genital schistosomiasis due to Schistosoma haematobium.

Clinical and parasitological findings in women in rural Malawi. *Acta Trop* (1996) 62(4):239–55. doi: 10.1016/s0001-706x(96)00026-5
8. Masson L, Passmore JA, Liebenberg LJ, Werner L, Baxter C, Arnold KB, et al. Genital inflammation and the risk of HIV acquisition in women. *Clin Infect Dis* (2015) 61(2):260–9. doi: 10.1093/cid/civ298
9. Passmore JA, Jaspan HB, Masson L. Genital inflammation, immune activation and risk of sexual HIV acquisition. *Curr Opin HIV AIDS* (2016) 11(2):156–62. doi: 10.1097/COH.0000000000000232
10. Osborn L, Kunkel S, Nabel GJ. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. *Proc Natl Acad Sci U S A* (1989) 86(7):2336–40. doi: 10.1073/pnas.86.7.2336
11. Masson L, Mlisana K, Little F, Werner L, Mkhize NN, Ronacher K, et al. Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study. *Sex Transm Infect* (2014) 90(8):580–7. doi: 10.1136/sextrans-2014-051601
12. Arnold KB, Burgener A, Birse K, Romas L, Dunphy LJ, Shahabi K, et al. Increased levels of inflammatory cytokines in the female reproductive tract are associated with altered expression of proteases, mucosal barrier proteins, and an influx of HIV-susceptible target cells. *Mucosal Immunol* (2016) 9(1):194–205. doi: 10.1038/mi.2015.51
13. Kyongo JK, Crucitti T, Menten J, Hardy L, Cools P, Michiels J, et al. Cross-Sectional Analysis of Selected Genital Tract Immunological Markers and Molecular Vaginal Microbiota in Sub-Saharan African Women, with Relevance to HIV Risk and Prevention. *Clin Vaccine Immunol* (2015) 22 (5):526–38. doi: 10.1128/CVI.00762-14
14. Mlisana K, Naicker N, Werner L, Roberts L, van Loggerenberg F, Baxter C, et al. Symptomatic vaginal discharge is a poor predictor of sexually transmitted infections and genital tract inflammation in high-risk women in South Africa. *J Infect Dis* (2012) 206(1):6–14. doi: 10.1093/infdis/jis298
15. Gosmann C, Anahtar MN, Handley SA, Farcasanu M, Abu-Ali G, Bowman BA, et al. Lactobacillus-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women. *Immunity* (2017) 46(1):29–37. doi: 10.1016/j.immuni.2016.12.013
16. Kyongo JK, Jespers V, Goovaerts O, Michiels J, Menten J, Fichorova RN, et al. Searching for lower female genital tract soluble and cellular biomarkers: defining levels and predictors in a cohort of healthy Caucasian women. *PLoS One* (2012) 7(8):e43951. doi: 10.1371/journal.pone.0043951
17. Francis SC, Hou Y, Baisley K, van de Wijgert J, Watson-Jones D, Ao TT, et al. Immune Activation in the Female Genital Tract: Expression Profiles of Soluble Proteins in Women at High Risk for HIV Infection. *PLoS One* (2016) 11(1):e0143109. doi: 10.1371/journal.pone.0143109
18. Al-Harthi L, Kovacs A, Coombs RW, Reichelderfer PS, Wright DJ, Cohen MH, et al. A menstrual cycle pattern for cytokine levels exists in HIV-positive women: implication for HIV vaginal and plasma shedding. *AIDS* (2001) 15 (12):1535–43. doi: 10.1097/00002030-200108170-00011
19. Lyke KE, Dabo A, Sangare L, Arama C, Daou M, Diarra I, et al. Effects of concomitant Schistosoma haematobium infection on the serum cytokine levels elicited by acute Plasmodium falciparum malaria infection in Malian children. *Infect Immun* (2006) 74(10):5718–24. doi: 10.1128/IAI.01822-05
20. Dupnik KM, Lee MH, Mishra P, Reust MJ, Colombe S, Haider SR, et al. Altered cervical mucosal gene expression and lower IL-15 levels in women with S. haematobium but not S. mansoni infection. *J Infect Dis* (2019) 219 (11):1777–85. doi: 10.1093/infdis/jiy742
21. Leutscher PD, Pedersen M, Raharisolo C, Jensen JS, Hoffmann S, Lisse I, et al. Increased prevalence of leukocytes and elevated cytokine levels in semen from Schistosoma haematobium-infected individuals. *J Infect Dis* (2005) 191 (10):1639–47. doi: 10.1086/429334
22. Hayes RJ, Donnell D, Floyd S, Mandla N, Bwalya J, Sabapathy K, et al. Effect of Universal Testing and Treatment on HIV Incidence - HPTN 071 (PopART). *N Engl J Med* (2019) 381(3):207–18. doi: 10.1056/NEJMoa1814556
23. Sturt AS, Webb EL, Phiri CR, Mweene T, Chola N, van Dam GJ, et al. Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. *PLoS Negl Trop Dis* (2020) 14(7):e0008337. doi: 10.1371/journal.pntd.0008337
24. World Health Organization. "Female genital schistosomiasis: a pocket atlas for clinical health-care professionals", in: *World Health Organization* (2015). Available at: http://www.who.int/iris/handle/10665/180863 (Accessed November 2, 2020).
25. Zambian Ministry of Health. Guidelines for the Etiological and Clinical Management of Sexually Transmitted Infections in Zambia. (2017) 1–48.
26. Corstjens PL, De Dood CJ, Kornelis D, Fat EM, Wilson RA, Kariuki TM, et al. Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology* (2014) 141(14):1841–55. doi: 10.1017/S0031182014000626
27. van Lieshout L, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. *Acta Trop* (2000) 77(1):69–80. doi: 10.1016/s0001-706x(00)00115-7
28. Corstjens P, de Dood CJ, Knopp S, Clements MN, Ortu G, Umulisa I, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Schistosoma Infections-Improvement and Use during SCORE. *Am J Trop Med Hyg* (2020) 103(1_Suppl):50–7. doi: 10.4269/ajtmh.19-0819
29. Obeng BB, Aryeetey YA, de Dood CJ, Amoah AS, Larbi IA, Deelder AM, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. *Ann Trop Med Parasitol* (2008) 102(7):625–33. doi: 10.1179/136485908X337490
30. Pillay P, Taylor M, Zulu SG, Gundersen SG, Verweij JJ, Hoekstra P, et al. Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. *Am J Trop Med Hyg* (2014) 90(3):546–52. doi: 10.4269/ajtmh.13-0406
31. Jespers V, van de Wijgert J, Cools P, Verhelst R, Verstraelen H, Delany-Moretlwe S, et al. The significance of Lactobacillus crispatus and L. vaginalis for vaginal health and the negative effect of recent sex: a cross-sectional descriptive study across groups of African women. *BMC Infect Dis* (2015) 15:115. doi: 10.1186/s12879-015-0825-z
32. Jiang Y, Zhang L, Kong F, Zhang M, Lv H, Liu G, et al. MCPerm: a Monte Carlo permutation method for accurately correcting the multiple testing in a meta-analysis of genetic association studies. *PLoS One* (2014) 9(2):e89212. doi: 10.1371/journal.pone.0089212
33. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. *Nat Rev Immunol* (2011) 11(6):375–88. doi: 10.1038/nri2992
34. Mutapi F, Winborn G, Midzi N, Taylor M, Mduluza T, Maizels RM. Cytokine responses to Schistosoma haematobium in a Zimbabwean population: contrasting profiles for IFN-gamma, IL-4, IL-5 and IL-10 with age. *BMC Infect Dis* (2007) 7:139. doi: 10.1186/1471-2334-7-139
35. Fu CL, Odegaard JI, Herbert DR, Hsieh MH. A novel mouse model of Schistosoma haematobium egg-induced immunopathology. *PLoS Pathog* (2012) 8(3):e1002605. doi: 10.1371/journal.ppat.1002605
36. van den Biggelaar AH, Borrmann S, Kremsner P, Yazdanbakhsh M. Immune responses induced by repeated treatment do not result in protective immunity to Schistosoma haematobium: interleukin (IL)-5 and IL-10 responses. *J Infect Dis* (2002) 186(10):1474–82. doi: 10.1086/344352
37. Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol* (2000) 164 (5):2585–91. doi: 10.4049/jimmunol.164.5.2585
38. Amiri P, Locksley RM, Parslow TG, Sadick M, Rector E, Ritter D, et al. Tumour necrosis factor alpha restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice. *Nature* (1992) 356(6370):604–7. doi: 10.1038/356604a0
39. King CL, Malhotra I, Mungai P, Wamachi A, Kioko J, Muchiri E, et al. Schistosoma haematobium-induced urinary tract morbidity correlates with increased tumor necrosis factor-alpha and diminished interleukin-10 production. *J Infect Dis* (2001) 184(9):1176–82. doi: 10.1086/323802
40. Chehimi J, Marshall JD, Salvucci O, Frank I, Chehimi S, Kawecki S, et al. IL-15 enhances immune functions during HIV infection. *J Immunol* (1997) 158 (12):5978–87.
41. Dessein A, Kouriba B, Eboumbou C, Dessein H, Argiro L, Marquet S, et al. Interleukin-13 in the skin and interferon-gamma in the liver are key players in immune protection in human schistosomiasis. *Immunol Rev* (2004) 201:180–90. doi: 10.1111/j.0105-2896.2004.00195.x

42. Mutengo MM, Mduluza T, Kelly P, Mwansa JCL, Kwenda G, Musonda P, et al. Low IL-6, IL-10, and TNF-alpha and High IL-13 Cytokine Levels Are Associated with Severe Hepatic Fibrosis in Schistosoma mansoni Chronically Exposed Individuals. *J Parasitol Res* (2018) 2018:9754060. doi: 10.1155/2018/9754060
43. Pearce EJ, MacDonald AS. The immunobiology of schistosomiasis. *Nat Rev Immunol* (2002) 2(7):499–511. doi: 10.1038/nri843
44. Marsden V, Donaghy H, Bertram KM, Harman AN, Nasr N, Keoshkerian E, et al. Herpes simplex virus type 2-infected dendritic cells produce TNF-alpha, which enhances CCR5 expression and stimulates HIV production from adjacent infected cells. *J Immunol* (2015) 194(9):4438–45. doi: 10.4049/jimmunol.1401706
45. Yegorov S, Joag V, Galiwango RM, Good SV, Mpendo J, Tannich E, et al. Schistosoma mansoni treatment reduces HIV entry into cervical CD4+ T cells and induces IFN-I pathways. *Nat Commun* (2019) 10(1):2296. doi: 10.1038/s41467-019-09900-9
46. Dupnik KM, Reust MJ, Vick KM, Yao B, Miyaye D, Lyimo E, et al. Gene Expression Differences in Host Response to Schistosoma haematobium Infection. *Infect Immun* (2019) 87(1):e00291–18. doi: 10.1128/IAI.00291-18
47. Kjetland EF, Ndhlovu PD, Mduluza T, Gomo E, Gwanzura L, Mason PR, et al. Simple clinical manifestations of genital Schistosoma haematobium infection in rural Zimbabwean women. *Am J Trop Med Hyg* (2005) 72(3):311–9. doi; 10.4269/ajtmh.2005.72.311
48. Pillay P, van Lieshout L, Taylor M, Sebitloane M, Zulu SG, Kleppa E, et al. Cervical cytology as a diagnostic tool for female genital schistosomiasis: Correlation to cervical atypia and Schistosoma polymerase chain reaction. *CytoJournal* (2016) 13:10. doi: 10.4103/1742-6413.180784
49. Herold BC, Keller MJ, Shi Q, Hoover DR, Carpenter CA, Huber A, et al. Plasma and mucosal HIV viral loads are associated with genital tract inflammation in HIV-infected women. *J Acquir Immune Defic Syndr* (2013) 63(4):485–93. doi: 10.1097/QAI.0b013e3182961cfc
50. Mave V, Erlandson KM, Gupte N, Balagopal A, Asmuth DM, Campbell TB, et al. Inflammation and Change in Body Weight With Antiretroviral Therapy Initiation in a Multinational Cohort of HIV-Infected Adults. *J Infect Dis* (2016) 214(1):65–72. doi: 10.1093/infdis/jiw096
51. Archary D, Liebenberg LJ, Werner L, Tulsi S, Majola N, Naicker N, et al. Randomized Cross-Sectional Study to Compare HIV-1 Specific Antibody and Cytokine Concentrations in Female Genital Secretions Obtained by Menstrual Cup and Cervicovaginal Lavage. *PLoS One* (2015) 10(7):e0131906. doi: 10.1371/journal.pone.0131906
52. Hegertun IE, Sulheim Gundersen KM, Kleppa E, Zulu SG, Gundersen SG, Taylor M, et al. S. haematobium as a common cause of genital morbidity in girls: a cross-sectional study of children in South Africa. *PLoS Negl Trop Dis* (2013) 7(3):e2104. doi: 10.1371/journal.pntd.0002104
53. Kjetland EF, Mduluza T, Ndhlovu PD, Gomo E, Gwanzura L, Midzi N, et al. Genital schistosomiasis in women: a clinical 12-month in vivo study following treatment with praziquantel. *Trans R Soc Trop Med Hyg* (2006) 100(8):740–52. doi: 10.1016/j.trstmh.2005.09.010

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

S1 Table – Crude concentrations of eleven cytokines and six chemokines in the cervicovaginal lavage of 212 Zambian women

Analyte	% (n) Above LLOQ	Median (IQR) * concentration	Mean (SD) * concentration	LLOQ Serum	LLOQ CVL
Eotaxin	73.6 (156)	4.83 (3.60 – 6.64)	5.22 (2.46)	4.0	0.98
Interferon–γ	84.0 (178)	1.03 (0.70 – 1.67)	2.03 (4.44)	0.8	0.33
IL–10	90.1 (191)	1.21 (0.75 – 2.55)	2.53 (6.00)	1.1	0.33
IL–13	32.6 (69)	0.76 (0.49 – 1.35)	1.10 (1.09)	1.3	0.31
IL–15	47.2 (100)	0.75 (0.48 – 1.24)	1.09 (1.10)	1.2	0.34
IL–17A	93.4 (198)	1.52 (0.92 – 3.04)	4.32 (14.64)	0.7	0.26
IL1–α	99.5 (211)	50.00 (19.89 – 164.86)	225.99 (551.30)	9.4	0.97
IL1–β	93.4 (198)	13.05 (2.88 – 72.83)	161.49 (414.08)	0.8	0.35
IL–4	96.2 (204)	2.87 (1.63 – 5.03)	3.86 (3.49)	4.5	0.31
IL–5	17.0 (36)	0.50 (0.38 – 0.72)	0.64 (0.54)	0.5	0.32
IL–6	87.7 (186)	4.09 (1.56 – 13.66)	13.54 (43.36)	0.9	0.32
IL–8	100.0 (212)	531.22 (230.01 – 1268.70)	1034.25 (1261.70)	0.4	5.66
IP–10	100.0 (212)	101.74 (32.66 – 276.30)	393.23 (1143.74)	8.6	1.37
MCP–1	100.0 (212)	27.84 (8.15 – 72.09)	76.83 (191.26)	1.9	1.03
MIP1–α	98.1 (208)	4.27 (2.12 – 15.04)	13.18 (27.32)	2.9	0.55
MIP1–β	83.5 (177)	2.02 (0.85 – 5.44)	6.59 (19.29)	3.0	0.28
TNF–α	41.0 (87)	1.18 (0.53 – 3.77)	4.99 (13.04)	0.7	0.32

Abbreviations: IQR – interquartile range, LLOQ – lower limit of quantification, SD – standard deviation, CVL – cervicovaginal lavage

* Concentrations are reported in pg/mL

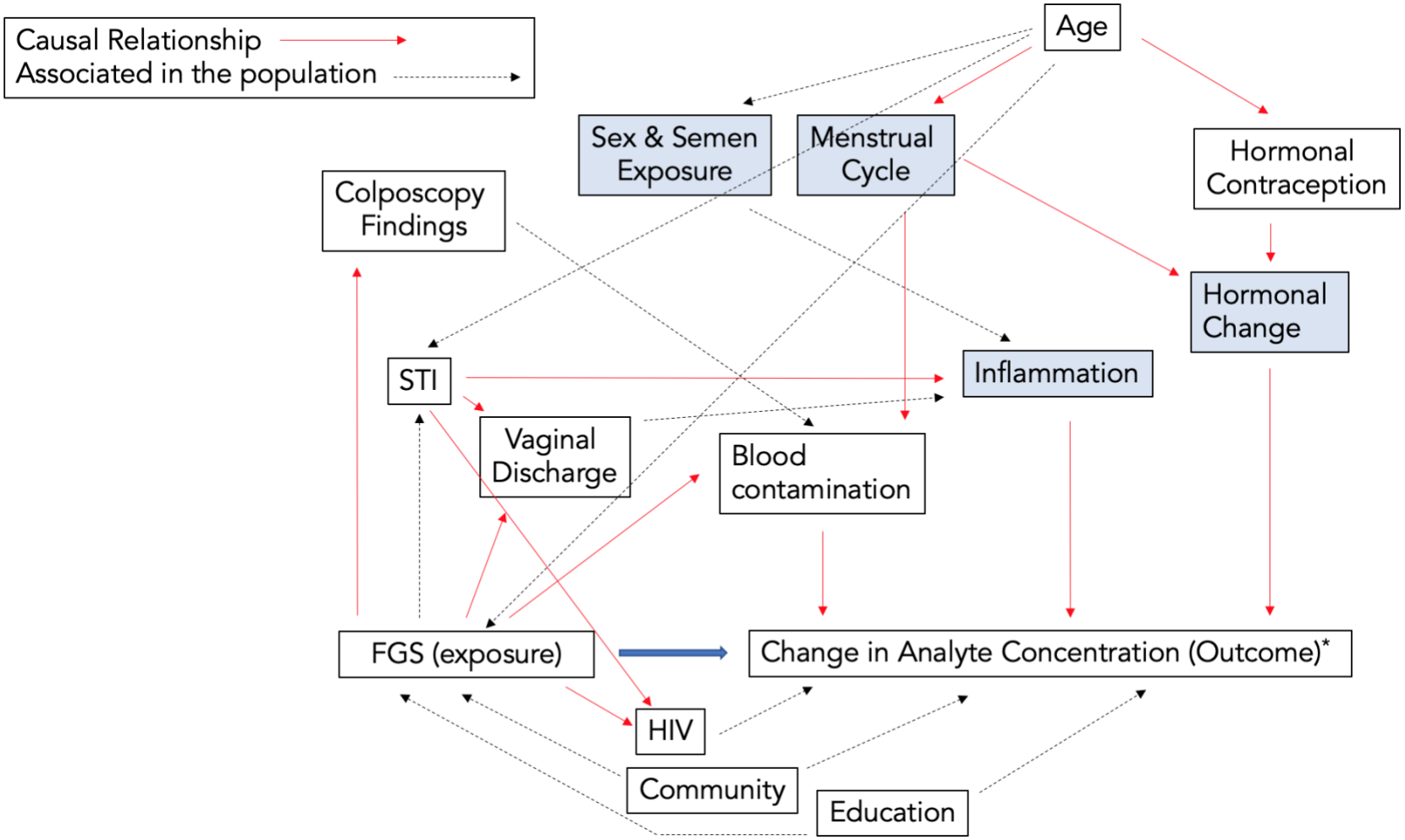
This table shows the concentrations of soluble immune proteins prior to imputation for those analytes below the limit of quantification.

S2 Table – Principal component analysis on data from log-transformed cytokines and chemokines

	Comp1	Comp2	Comp3	Comp4	Comp5	Comp6	Comp7	Comp8	Comp9	Comp10	Comp11	Comp12	Comp13	Comp14	Comp15	Comp16	Comp17
Eotaxin	0.099	0.597	-0.123	0.295	-0.343	0.423	0.214	-0.098	-0.132	0.266	0.196	0.011	0.008	0.099	0.012	0.201	0.051
INF–γ	0.258	0.154	-0.158	-0.234	-0.099	-0.119	-0.585	-0.149	0.147	0.364	0.017	-0.159	-0.241	-0.095	0.432	-0.048	-0.072
IL–10	0.295	0.026	-0.021	-0.094	-0.232	-0.272	-0.170	-0.023	0.166	-0.110	-0.197	0.038	-0.023	0.049	-0.453	0.652	0.181
IL–13	0.262	-0.020	-0.262	-0.334	-0.003	0.091	0.228	-0.153	-0.105	-0.285	-0.165	0.200	0.480	-0.066	0.472	0.207	-0.088
IL–15	0.268	0.284	-0.205	-0.074	0.202	0.366	-0.254	0.026	-0.340	-0.246	-0.074	-0.528	0.063	0.091	-0.330	-0.098	-0.224
IL–17a	0.248	0.225	-0.076	0.261	0.140	-0.178	-0.257	0.583	-0.370	-0.132	-0.043	0.402	-0.023	-0.086	0.089	-0.089	0.127
IL1–α	0.206	-0.212	-0.336	0.333	0.430	0.078	-0.044	-0.052	0.349	0.211	0.387	0.106	0.244	-0.265	-0.135	0.129	-0.043
IL1–β	0.265	-0.284	0.008	0.299	0.182	0.064	0.180	0.102	0.065	0.093	-0.219	-0.287	-0.146	0.466	0.348	0.114	0.407
IL–4	0.260	0.310	-0.075	0.161	-0.151	-0.075	0.102	-0.003	0.571	-0.293	-0.264	-0.075	0.105	-0.043	-0.087	-0.506	0.050
IL–5	0.225	0.014	-0.278	-0.437	0.034	-0.083	0.521	0.373	0.046	0.132	0.175	-0.085	-0.420	-0.118	-0.098	-0.037	-0.061
IL–6	0.246	-0.175	0.367	0.029	-0.076	0.239	0.098	0.155	-0.041	0.478	-0.492	0.029	0.148	-0.381	-0.091	-0.028	-0.169
IL–8	0.259	0.089	0.143	0.247	0.269	-0.157	0.191	-0.541	-0.211	-0.190	-0.108	0.119	-0.474	-0.218	0.030	0.037	-0.189
IP–10	0.163	0.395	0.369	-0.221	0.420	-0.351	0.107	-0.072	-0.060	0.262	0.132	-0.111	0.360	0.271	-0.093	-0.053	0.019
MCP–1	0.190	0.036	0.464	-0.285	0.168	0.535	-0.151	0.051	0.254	-0.250	0.243	0.249	-0.185	0.007	0.033	0.044	0.204
MIP1–α	0.267	-0.188	0.264	0.196	-0.293	-0.126	0.017	0.188	0.099	-0.118	0.305	-0.010	0.027	0.298	0.126	0.071	-0.649
MIP1–β	0.266	-0.200	0.198	0.040	-0.344	-0.166	0.051	-0.098	-0.252	-0.103	0.407	-0.288	0.163	-0.374	0.030	-0.157	0.420
TNF–α	0.266	-0.275	-0.179	-0.128	-0.174	0.046	-0.041	-0.286	-0.169	0.222	0.026	0.463	-0.003	0.403	-0.277	-0.385	0.092
POV	0.519	0.087	0.081	0.062	0.046	0.037	0.027	0.024	0.021	0.018	0.017	0.015	0.013	0.011	0.011	0.007	0.005
CPOV	0.519	0.606	0.687	0.749	0.795	0.831	0.856	0.882	0.903	0.921	0.939	0.954	0.966	0.977	0.989	0.995	1.000

Abbreviations: Comp – component, CPOV – cumulative proportion of variance explained, IL–interleukin, INF –γ Interferon-γ, MCP1 – monocyte chemoattractant protein-1, MIP1-α – macrophage inflammatory protein 1–α, MIP1-β – macrophage inflammatory protein 1-β, POV – proportion of variance explained, TNF-α – Tumor necrosis factor–α

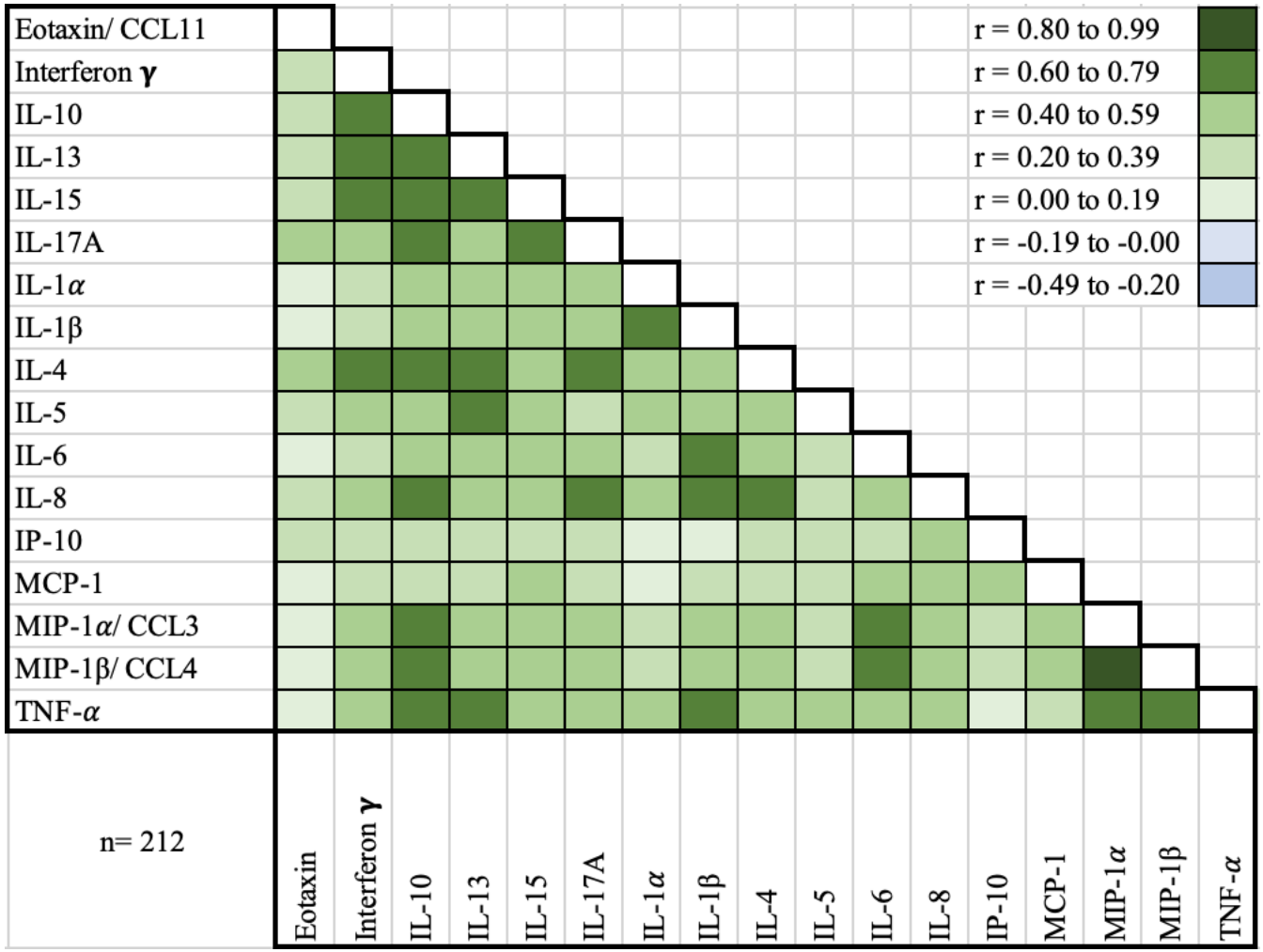
S1 Figure – Causal diagram for the association between FGS and a concentration change of cytokines and chemokines



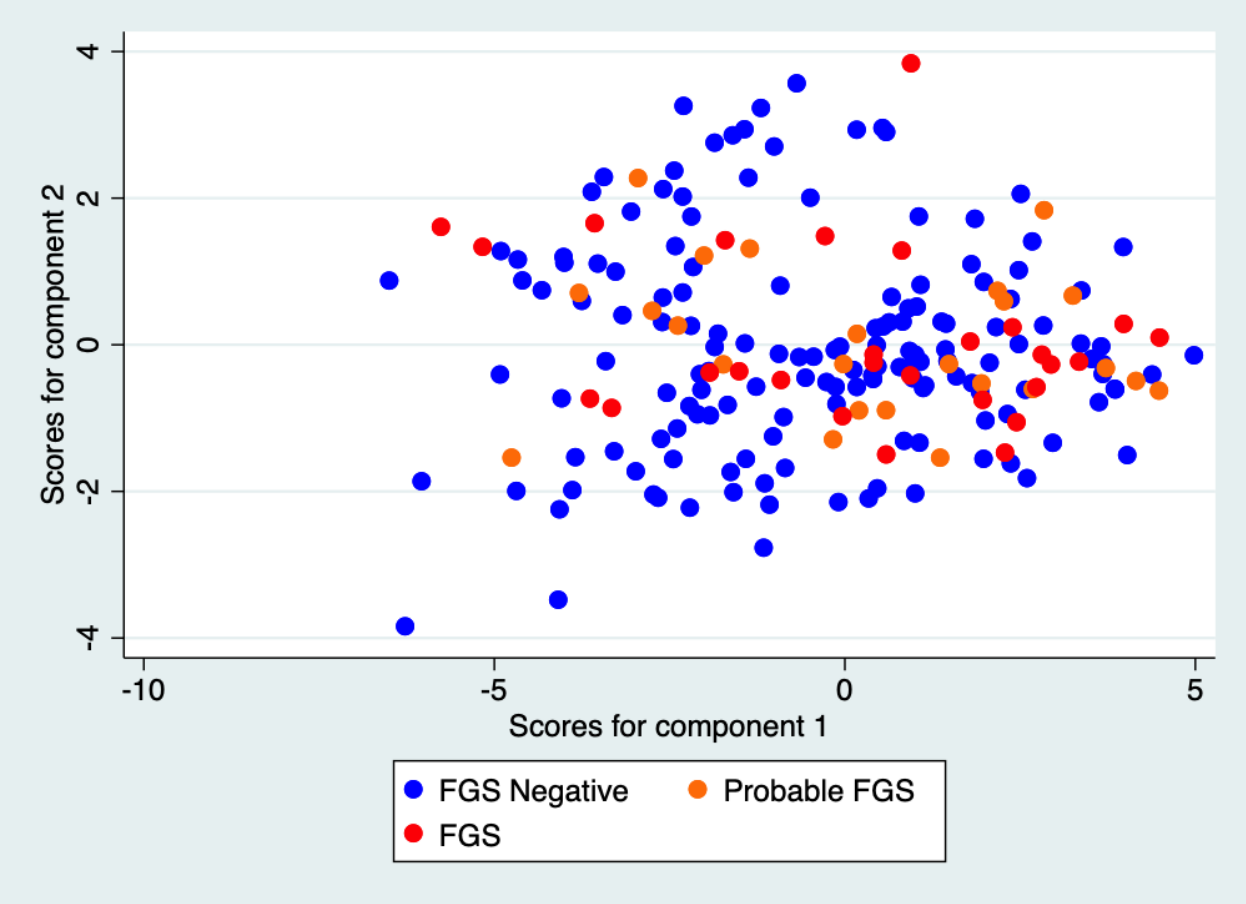
Shaded items were not measured objectively.

Adapted from Francis SC, et al. Immune Activation in the Female Genital Tract: Expression Profiles of Soluble Proteins in Women at High Risk for HIV Infection. *PloS one* (2016) 11(1):e0143109.

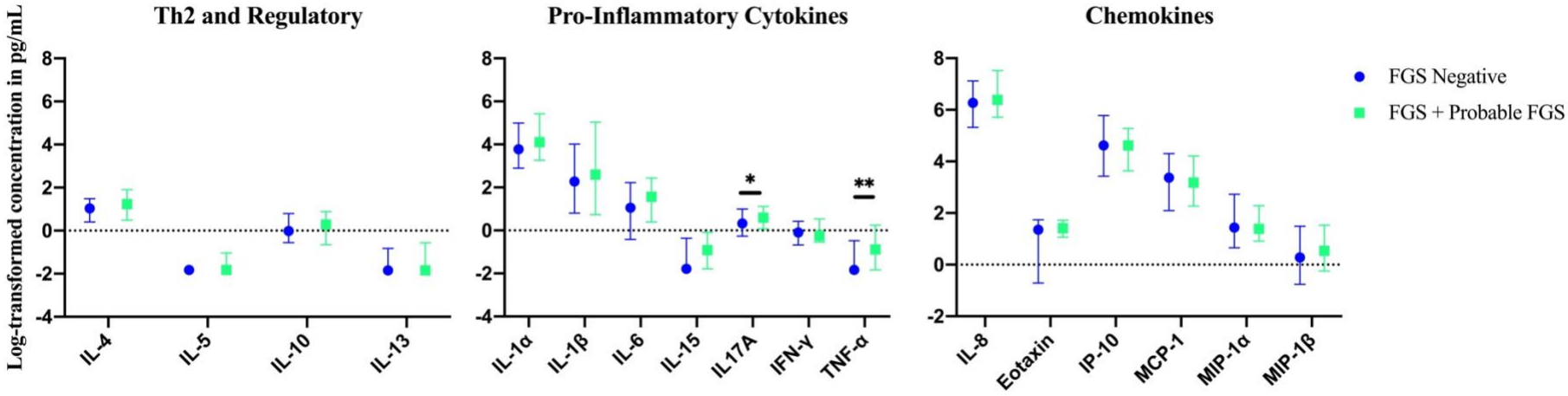
S2 Figure – Spearman's Rank Correlations by Cytokine and Chemokine Panel



S3 Figure – Scatterplot of the first two principal component scores by FGS Status



S4 Figure – Median with interquartile range of the log-transformed crude concentrations of eleven cytokines and six chemokines in cervicovaginal lavage of participants with FGS and Probable FGS (n=53) and without female genital schistosomiasis (FGS negative: n=159)ᵡ



S4 Figure Caption

ᵡFGS – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative genital PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging
p-value after adjustment for multiple testing with a Monte-Carlo simulation approach, p=0.09

p-value symbol legend:
*p<0.1
**p<0.05

S5 Figure – Comparison of the concentration or presence of eleven cytokines and six chemokines in cervicovaginal lavage in participants with (FGS + Probable FGS, n=53) and without (negative FGS, n=159) female genital schistosomiasis[χ]

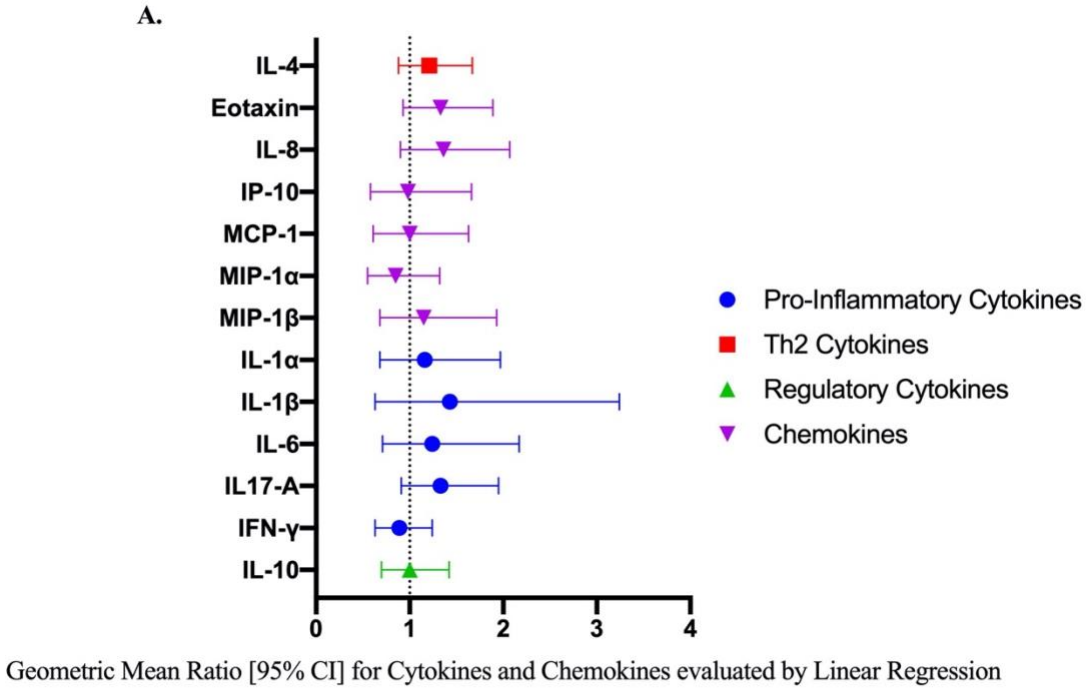


Figure S5 Caption

A. Concentrations of eotaxin, IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, MCP-1, MIP-1α, and MIP-1β were compared between FGS and FGS *negative* by linear regression adjusted for age, community of residence, education, presence of sexually transmitted infection and hormonal contraceptive use, with results shown as geometric mean ratios with 95% CI.

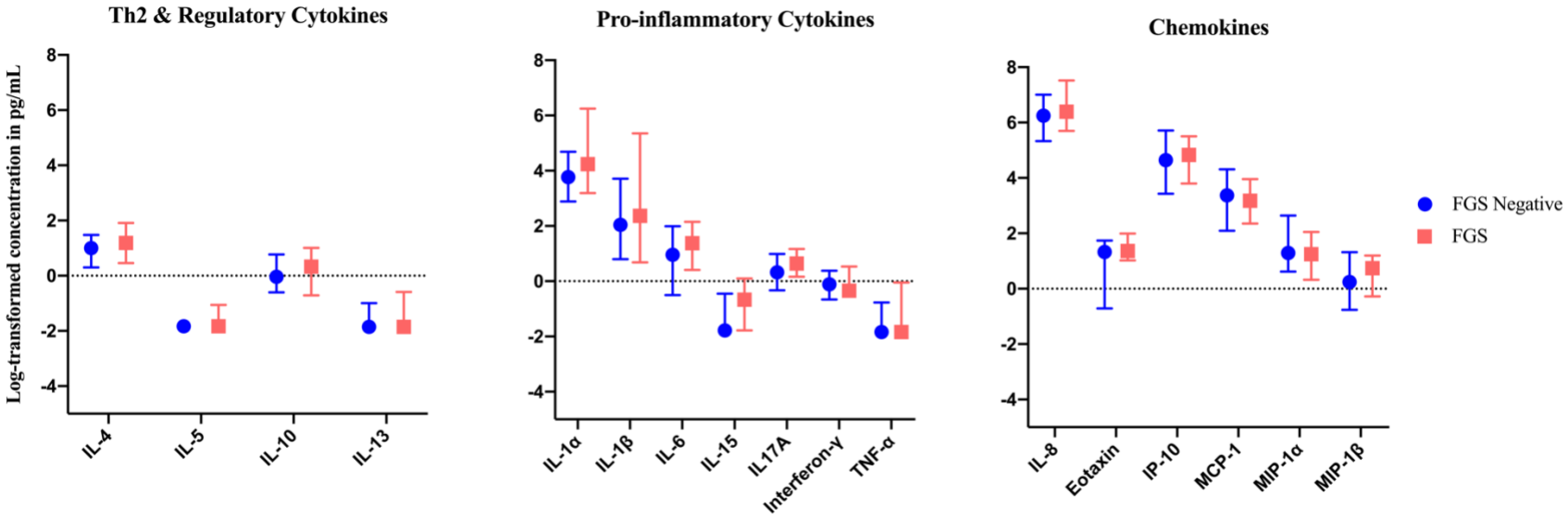
B. Presence/absence of IL-5, IL-13, IL-15 and TNF-α were compared by logistic regression and adjusted for age and sexually transmitted infection, with results shown as odds ratio with 95% CI.

The line at 1 indicates the value at which there is no difference between the FGS and *negative* FGS groups

p-value symbol legend:
**p<0.05

ɤfemale genital schistosomiasis – *Schistosoma* PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative genital PCR and negative urine circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging

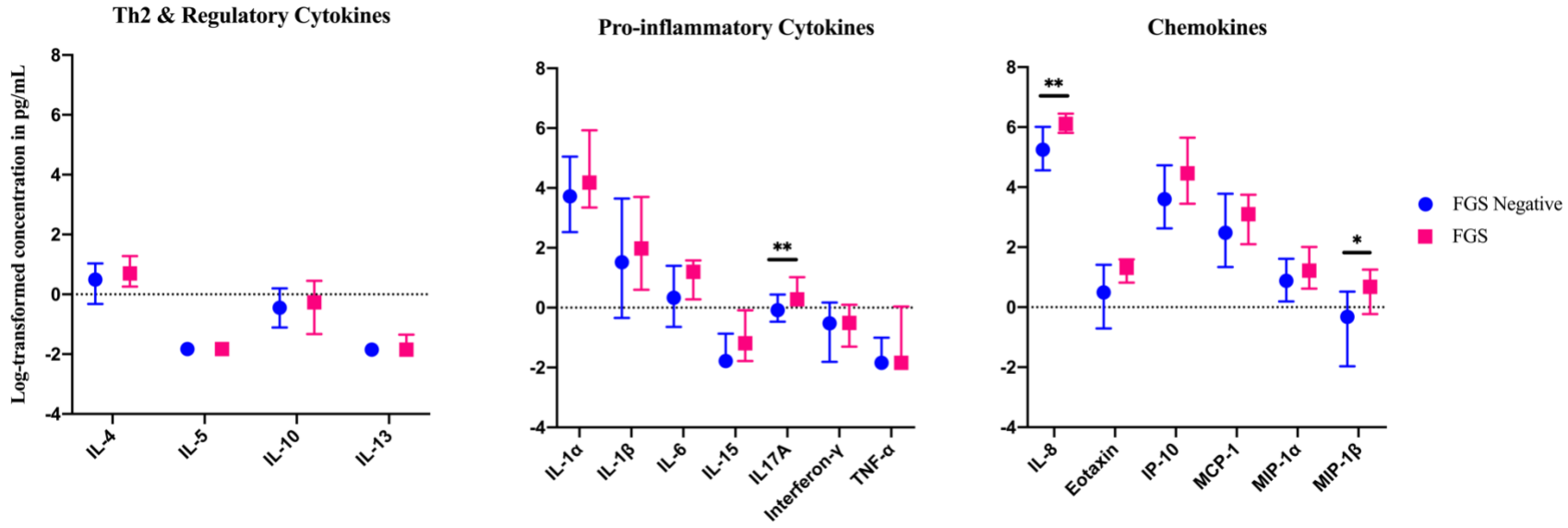
S6 Figure – Sensitivity analysis: Median with interquartile range of the log-transformed concentrations of eleven cytokines and six chemokines in cervicovaginal lavage by FGS Status (FGS: n=23, FGS negative: n=133), participants with HIV-1 excluded[χ]



[χ]FGS – PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative *Schistosoma* PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging

All p-values are greater than 0.1

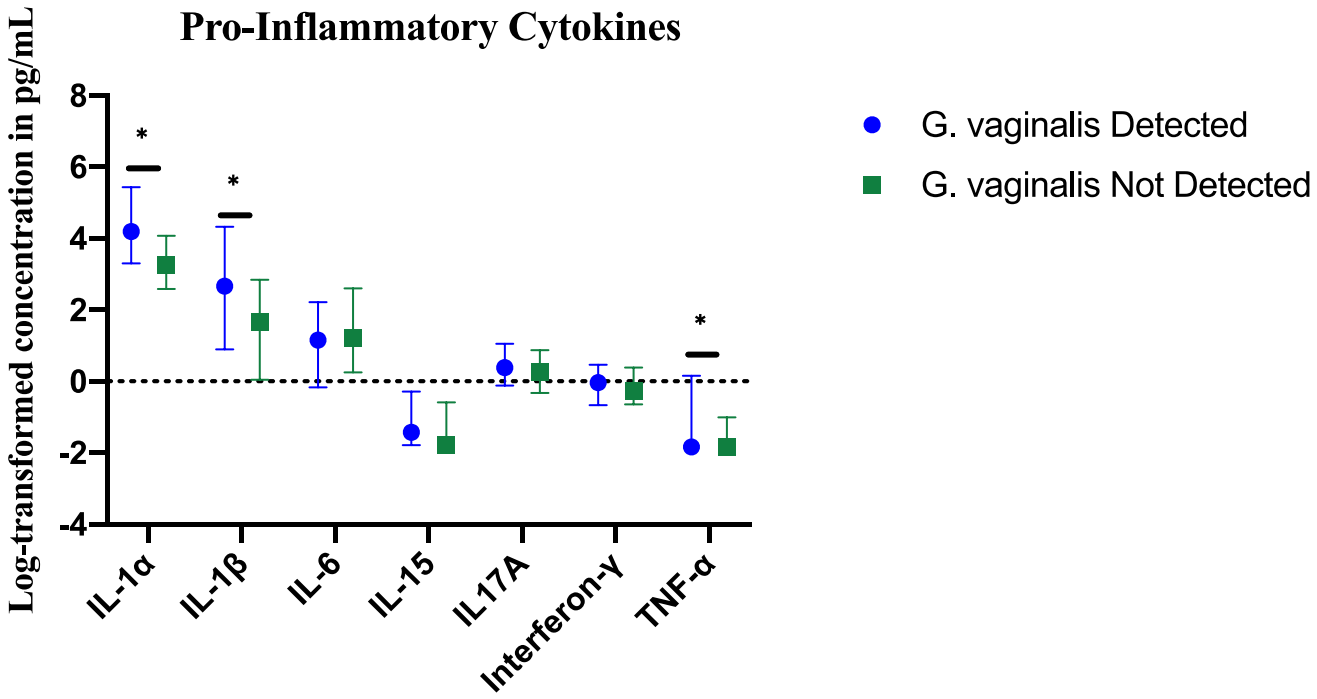
S7 Figure – Median with interquartile range of the log-transformed crude concentrations of eleven cytokines and six chemokines in cervicovaginal lavage of participants with (FGS: n=28) and without female genital schistosomiasis (FGS negative: n=159)



ɤFGS – PCR positive specimen from cervicovaginal lavage, vaginal swab or cervical swab; FGS *negative* – negative *Schistosoma* PCR and negative circulating anodic antigen and negative urine microscopy and negative expert-reviewed colposcopy imaging
p-value after adjustment for multiple testing with a Monte-Carlo simulation approach, p=0.13

p-value symbol legend:
*p<0.1
** p<0.05

S8 Figure – Median with interquartile range of the log-transformed crude concentrations of seven pro-inflammatory cytokines in cervicovaginal lavage by *Gardnerella vaginalis* status



S8 Figure Caption

p-value symbol legend:

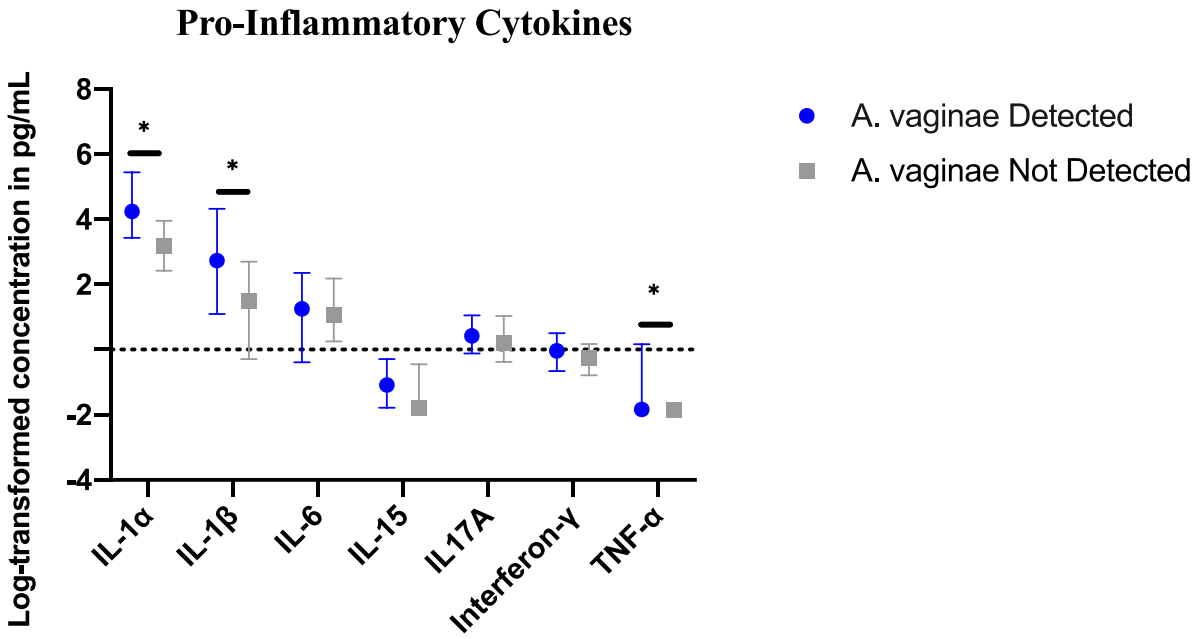
*p<0.05 , IL-1α, IL-1β,

IL-1α – Rank-sum p-value <0.001 (for the comparison of concentrations by presence/absence of *G. vaginalis*); Kruskal Wallis p-value, p=0.002 (for the comparison of concentrations by *G. vaginalis* density)

IL-1β – Rank-sum p-value 0.005 (for the comparison of concentrations by presence/absence of *G. vaginalis*); Kruskal Wallis p-value, p=0.02 (for the comparison of concentrations by *G. vaginalis* density)

TNF-α – Rank-sum p-value 0.04 (for the comparison of concentrations by presence/absence of *G. vaginalis*); Kruskal Wallis p-value, p=0.04 (for the comparison of concentrations by *G. vaginalis* density)

S9 Figure– Median with interquartile range of the log-transformed crude concentrations of seven pro-inflammatory cytokines in cervicovaginal lavage by *Atopobium vaginae* status



S9 Figure Caption

p-value symbol legend:

*p<0.05

IL-1α – Rank-sum p-value <0.001 (for the comparison of concentrations by presence/absence of *A. vaginae*); Kruskal Wallis p-value, p<0.001 (for the comparison of concentrations by *A. vaginae* density)

IL-1β – Rank-sum p-value <0.001 (for the comparison of concentrations by presence/absence of *A. vaginae*); Kruskal Wallis p-value, p<0.001 (for the comparison of concentrations by *A. vaginae* density)

TNF-α – Rank-sum p-value 0.002 (for the comparison of concentrations by presence/absence of *A. vaginae*); Kruskal Wallis p-value, p=0.02 (for the comparison of concentrations by *A. vaginae* density)

Chapter 7

Research paper 4

Chapter 7 – Female genital schistosomiasis and HIV-1 incidence in Zambian women: a retrospective cohort study

Citation: Sturt AS, Webb EL, Phiri C, Mudenda M, Mapani J, et al. Female genital schistosomiasis and HIV-1 incidence in Zambian women: a retrospective cohort study.

Accepted at Open Forum Infectious Diseases:
https://academic.oup.com/ofid/advance-article/doi/10.1093/ofid/ofab349/6311690

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Surname/Family Name	Sturt		
Thesis Title	Female Genital Schistosomiasis and HIV-1 incidence in Zambian women: a retrospective cohort study		
Primary Supervisor	Amaya Bustinduy		

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Date	July 16, 2021

Supervisor Signature	Amaya Bustinduy
Date	July 16, 2021

Female Genital Schistosomiasis and HIV-1 incidence in Zambian women: a retrospective cohort study

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Running Head: FGS and HIV-1 Incidence

Word Count: 3499/3500

Key Points: FGS has been associated with prevalent HIV-1. In this study, women with FGS had higher rates of HIV-1 seroconversion, however there was no statistical evidence of an association.

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Background:

Female genital schistosomiasis (FGS) has been associated with prevalent HIV-1. We estimated the incidence of HIV-1 infection in Zambian women with and without FGS.

Methods:

Women (aged 18-31, non-pregnant, sexually active) were invited to participate in this study in January-August 2018 at final follow-up of the HPTN 071 (PopART) Population Cohort. HIV-1 negative participants at enrolment (n=492) were included in this analysis with testing to confirm incident HIV-1 performed in HPTN 071 (PopART). Association of incident HIV-1 infection with FGS (*Schistosoma* DNA detected by PCR in any genital specimen) was assessed with exact Poisson regression.

Results:

Incident HIV-1 infections were observed in 4.1% (20/492) participants. Women with FGS were twice as likely to seroconvert as women without FGS but with no statistical evidence for a difference (aRR 2.16, 95%CI[0.21–12.30], p=0.33). Exploratory analysis suggested an association with HIV-1 acquisition among women with ≥2 positive genital PCR specimens (RR 6.02, [0.58–34.96]), p=0.13).

Conclusions:

Despite higher HIV seroconversion rates in women with FGS, there was no statistical evidence of association, possibly due to low power. Further longitudinal studies should investigate this association in a setting with higher schistosomiasis endemicity.

Keywords: HIV incidence, female genital schistosomiasis, polymerase chain reaction, PCR, parasite, *Schistosoma haematobium*

Introduction

In 2019, an estimated 56 million women were living with female genital schistosomiasis (FGS), a neglected tropical disease that results when eggs from the parasite *Schistosoma (S.) haematobium* are deposited in reproductive tract tissues [1]. Tissue-entrapped eggs incite a cellular response [2], ultimately resulting in FGS-related morbidity, including infertility [3], and distinct cervicovaginal manifestations [4, 5]. In sub-Saharan Africa, there is a geographical association between areas of high *S. haematobium* prevalence and HIV-1 infection [6], and FGS has been associated with prevalent HIV-1 [7] with biological plausibility for a causal relationship [3, 8]. Despite global advances in HIV-1 treatment and prevention, gender related disparities still exist, with particularly heightened risk among young women aged 15-24 years [9]. HIV-1 vulnerability in young women is multifactorial, including biological, behavioural, demographic, social, and structural components [10]. The potential role of FGS as an underreported and preventable co-factor in HIV-1 vulnerability needs further investigation.

Disruption of the protective vaginal and cervical epithelium by FGS-associated lesions may increase HIV-1 susceptibility by providing a portal for viral entry [3, 11]. Additionally, the environment created by *S. haematobium* eggs is more vascular [12], with an increased density of CD4+ lymphocytes [8] compared to non-egg containing tissue. Thus, tissue-entrapped *S. haematobium* eggs create a cellular milieu which may promote HIV-1 infection. Both *S. haematobium and S. mansoni* infection have been associated with prevalent HIV-1 [11]. A cross-sectional study of women with FGS, defined as parasite eggs detected in genital tissue, describes a strong association with prevalent HIV-1 but no evidence of an association between urinary schistosomiasis and HIV-1 [7]. Studies primarily evaluating urinary *S. haematobium* (without

universal evaluation for genital involvement) and prevalent HIV-1 have been mixed, with evidence of an association with prevalent HIV-1 in a study of Tanzanian women [13], some evidence of an association with prevalent HIV-1 in a study of Zimbabwean women [14], but with no evidence of an association with prevalent HIV-1 in men and women with urinary *S. haematobium* in Congo [15]. While *S. haematobium* seropositivity in women has been associated with HIV-1 acquisition [16], the association of FGS with incident HIV-1 has not been described.

FGS diagnosis is challenging and its burden is likely underreported. The presence of parasite eggs or DNA in cervicovaginal tissue is diagnostic of FGS, [17, 18], and, historically, biopsy is used as a reference standard [4, 19]. However, theoretical concerns regarding post-biopsy HIV-1 acquisition has limited its acceptance in research settings [17]. Polymerase chain reaction (PCR) on cervicovaginal lavage (CVL) is a less-invasive means of FGS diagnosis, albeit with imperfect sensitivity [17, 18]. Well-defined clinical manifestations have been associated with FGS [5, 20] but are variably correlated with the presence of *S. haematobium* eggs [19, 20] or DNA [17] in genital tissue. The identification of clinical lesions, such as homogenous yellow sandy patches and abnormal blood vessels, with colposcopy is observer-dependent and subject to low specificity [21]. Indeed, homogeneous yellow sandy patches have also been associated with herpes simplex virus-2 and human papillomavirus, and abnormal blood vessels may be associated with cervical intraepithelial neoplasia [5]. Urine microscopy and circulating anodic antigen (CAA) can be used to detect active schistosome infection [18, 22], but do not assess involvement of genital tissue. While either *S. haematobium* and *S. mansoni* can cause FGS, the majority of cases are attributed to *S. haematobium* [23, 24] and the current study focuses on *S. haematobium*. We conducted an array of diagnostic tests for *S. haematobium* infection (CAA and urine microscopy) and FGS

(portable colposcopy, cervical swabs, vaginal swabs, and cervicovaginal lavage), and have previously demonstrated that self-collected genital swabs had comparable sensitivity to clinic-based, midwife-collected CVL for the detection of *Schistosoma* DNA by real-time PCR [18].

The longitudinal follow-up of women in the HPTN 071 (PopART) trial in two schistosomiasis-endemic communities in Zambia provided an opportunity for a nested study exploring the association of FGS with HIV-1 incidence.

Methods

Study setting and participants

The cross-sectional bilharzia and HIV (BILHIV) study was nested in HPTN 071 (PopART), a cluster randomized trial assessing the impact of an HIV-1 combination prevention package including "universal testing and treatment" [25]. HIV-1 incidence was measured in a Population Cohort (PC) comprised of one randomly selected adult (18 to 44 years of age) from a random sample of households in each community who provided data and blood samples at baseline, 12, 24, and 36 months [25]. Between January and August 2018, after the 36-month HPTN 071 (PopART) PC visit, trained community workers conducted home visits to women who had expressed interest in the BILHIV study [18]. Women in Livingstone, Zambia were eligible if they were 18-31 years old, not pregnant, sexually active, and residing in one of the two urban *S. haematobium* endemic communities that participated in one of two HPTN 071 (PopART) intervention arms.

Following written informed consent, the BILHIV study home visit included a questionnaire, genital self-sampling (cervical and vaginal), and a urine specimen, as previously described [18]. Within days of self-sampling, non-menstruating participants were invited to attend Livingstone Central Hospital for cervicovaginal lavage (CVL) [18]. Cervicovaginal images were captured with a portable colposcope (MobileODT, Tel Aviv, Israel) and evaluated by one author (EFK) for the presence of any of the four accepted FGS cervicovaginal manifestations: homogenous yellow sandy patches, grainy sandy patches, rubbery papules, and abnormal blood vessels [26]. Women with evidence of schistosome infection by colposcopy [26] or any positive urine or genital diagnostic were treated free of charge with 40 mg/kg praziquantel. Routine testing for sexually transmitted infections (STI) was not performed. Participants with suspected STI were offered syndromic management, as per local guidelines [27].

HIV-1

Laboratory-based fourth-generation HIV-1 testing (Abbott Architect HIV Ag/Ab ComboAssay, Wiesbaden, Germany) was performed for HPTN 071 (PopART) PC participants at each study visit [25]. Additional testing using antigen/antibody screening tests, a discriminatory test, and an HIV-1 RNA test was used to confirm incident HIV-1 infection, as previously described [28].

Circulating Anodic Antigen

CAA levels reflect the burden of live schistosomes and decline after successful treatment with praziquantel [22, 29]. An up-converting reporter particle lateral flow assay for the quantification of CAA in urine was performed at the Leiden University Medical Center (LUMC), as previously

described [18, 30]. Analysing the equivalent of 417 μL urine, a CAA value of >0.6 pg/mL was considered positive [22].

PCR for detection of Schistosoma DNA

DNA extraction and PCR was performed at LUMC as previously described, using a custom automated liquid handling station (Hamilton, Switzerland) [20, 31]. DNA was extracted from 200μL of specimen (cervical swab, vaginal swab, CVL) with QIAamp spin columns (QIAGEN Benelux; Venlo, The Netherlands). Detection of the schistosome-specific internal-transcribed-spacer-2 (ITS2) target was performed by real-time PCR as previously described [18, 31]. This PCR does not differentiate between *Schistosoma* species. DNA amplification and detection were performed with the CFX96 Real Time PCR Detection System (BioRad, California, USA). The output in threshold cycles (Ct), reflecting the parasite-specific DNA load in the tested sample, was analysed using BioRad CFX software. Parasite DNA loads were categorized by the following pre-specified C_t thresholds: high (C_t<30), moderate (30≤C_t<35), low (35≤ C_t <50) and negative (no C_t detected), as previously described [32].

Patient Consent Statement

The study was approved by the University of Zambia Biomedical Research Ethics Committee (reference 011-08-17), the Zambia National Health Research Authority and the London School of Hygiene and Tropical Medicine Ethics Committee (reference 14506). Permission to conduct the study was given by Livingstone District Health Office and the Livingstone Central Hospital superintendent. Each participant provided written informed consent.

FGS Definitions

Comparison groups were defined by the results of four investigations: genital PCR, colposcopy image review, urine CAA, and urine microscopy. Participants were grouped by the outcomes of their diagnostic tests into three mutually exclusive categories. *FGS* was defined as at least one positive genital PCR (cervical swab, vaginal swab or CVL) (Figure 1). *Probable/possible FGS* was defined as the presence of either a positive urine diagnostic (CAA or microscopy) or one of four cervicovaginal manifestations suggestive of FGS on portable colposcopy, or both, with negative genital PCR (Figure 1). FGS *negative* was defined as negative results on all diagnostics.

Statistical Methods

Characteristics of study participants were summarized by frequency and percentage. Women living with HIV-1 (WLHIV) at HPTN 071 (PopART) baseline were excluded from further analyses. HIV-1 incidence was calculated as the number of seroconversions per 1000 person-years of follow up. Participants contributed person-time for the calculation of HIV-1 incidence starting with their first HIV-1 test and ending at date of HIV-1 seroconversion for those who seroconverted, or at the date of last follow-up or the end of scheduled follow-up (whichever occurred earliest) for women who did not seroconvert. HIV-1 seroconversion was assumed to occur at the midpoint between the last negative and the first positive HIV-1 test. We assumed that FGS acquisition occurred prior to HPTN 071 (PopART) enrolment [33]. BILHIV study participants were consecutively recruited from the PC, providing the opportunity to compare the rate of incident HIV-1 infection in women with and without FGS, with power determined by the number of HIV-1 seroconversions and FGS prevalence. Data on HIV-1 outcomes were not available until after BILHIV study closure.

Associations of risk factors with incident HIV-1 infection were calculated as rate ratios and 95% confidence intervals, estimated using exact Poisson regression in univariable and multivariable analysis. We used a causal conceptual framework to inform our choice of potential confounders. *A priori,* we included age as a confounding variable. Due to loss of precision with further adjustment for potential confounding variables, no additional parameters were included in the multivariable model. To assess the primary exposure of interest, women with FGS (n=26) were compared with an FGS *negative* comparison group comprising those who were negative on all diagnostic investigations (n=218). Participants who were negative on all diagnostic investigations but missing colposcopy images (n=82) were excluded from the primary analysis.

To evaluate the association of schistosome infection intensity with HIV-1 seroconversion, two ad hoc exploratory analyses were performed. One compared participants with FGS and a moderate/high *Schistosoma* DNA concentration (Ct <35) with those in the FGS *negative* group. The second compared participants with FGS with ≥2 positive genital PCR specimens with those in the FGS *negative* group. Data were analysed using STATA 15.1 (Stata Corporation, College Station, TX).

Results

A total of 603 eligible women from the HPTN 071 Population Cohort were enrolled in the BILHIV study. WLHIV at HPTN 071 (PopART) trial entry (n=107, 17.7%) were excluded, with 492 (82.1%) included in this analysis (Figure 2). Of the included participants, 14% (69/492) did not attend clinic for CVL.

Baseline characteristics

The majority of participants had received at least secondary education, were not working, and reported being currently sexually active. A small proportion of women reported current water contact, but more than half reported childhood water contact. Active schistosome infection, defined as either a positive urine microscopy (5.5%, 27/492) or detectable CAA (15.1%, 74/492), was detected in 15.7% (77/492) of participants.

HIV incidence

The 492 women without HIV-1 at HPTN 071 (PopART) study entry provided a total of 1,164 person-years of follow-up, during which time, 20 (4.1%) incident HIV-1 infections were measured, for an overall rate of 17.2 (95% CI 11.1–26.6) seroconversions per 1000 person-years. HIV-1 incidence rates are shown by baseline characteristics in Table 1.

HIV-1 incidence rates were 23.6 (14.2 – 39.2) in women aged 18-24 years compared with 9.5 (3.9–22.7) in women aged 25-31, (RR 0.40 [0.15–1.10]), p=0.06 (Table 1)). The HIV-1 seroconversion rate decreased as the household size increased (p=0.007, test for trend) and increased as the number of lifetime sexual partners increased (p=0.01, test for trend). Women self-reporting a history of STI were more likely to seroconvert than women without self-reported STI (RR 5.76 [1.92–17.22], p=0.009) (Table 1). No other sociodemographic or behavioural characteristics were associated with HIV-1 incidence. After adjusting for age, there remained strong evidence that a higher number of people residing in a household (p=0.008, test for trend), a higher number of lifetime sexual partners (p=0.01, test for trend), and self-reported history of STI (aRR 6.05 [2.02–18.12], p=0.008) were associated with HIV-1 seroconversion. Additionally, there was no evidence for an association

between urinary schistosome infection (as defined by urine CAA and/or microscopy) and HIV-1 seroconversion (Table 2).

Association between FGS and HIV-1 seroconversion

FGS was identified in 5.3% of women (26/492), defined as any positive genital PCR (cervical swab 3.5%, [17/492]; vaginal swab 2.4%, [12/492]; or CVL 3.1%, [13/423]). Among women with a negative genital PCR, results from both urine and colposcopy imaging were positive in 4.5% (21/466) of participants and results from either urine or colposcopy imaging were positive in 31.1% (145/466). Of the participants with *probable/possible FGS*, 63.8% (106/166) had colposcopy changes in isolation (Figure 1), of whom 62.3% (66/106) had abnormal blood vessels and 37.7% (40/106) had grainy or homogenous yellow sandy patches on colposcopy. There were 218 (44.3%) participants who were negative on all diagnostic tests. The rate of HIV-1 seroconversion (per 1000 person/year) in women with FGS (31.0 [7.8 – 123.9]) was higher than in the FGS *negative* group (11.3[5.1–25.1]) (Table 2) but without statistical evidence of a difference between these rates in either univariable or multivariable analyses (crude RR 2.75 [0.27–15.36], p=0.26; aRR 2.16 (0.21–12.30), p=0.33) (Table 2).

Exploratory analyses:* Schistosoma *DNA concentrations and disease burden

In the ad hoc exploratory analysis of women (n=13) with FGS and moderate/high *Schistosoma* DNA concentrations the IRR for HIV-1 acquisition after adjusting for age was 4.73 (0.46–27.05), p=0.19) compared to FGS *negative* participants (Table 2). In an ad hoc exploratory analysis of women (n=13) with ≥2 positive genital PCR specimens compared to FGS *negative* participants, the IRR for HIV-1 acquisition after adjusting for age was 6.02 (0.58–34.96, p=0.13) (Table 2). In

these groups, n=9 of the women overlapped and the same two participants contributed seroconversions in both groups. There were no HIV-1 seroconversions in participants with one positive genital PCR.

Discussion

This study is the first to examine the association of PCR-defined FGS with HIV-1 incidence. While barriers to implementation still exist, PCR for *Schistosoma* DNA detection in FGS diagnosis is reproducible, has high specificity, and can be performed on self-collected genital specimens [18]. We found that women with FGS were twice as likely to seroconvert than women in the comparison group albeit with wide confidence intervals and no statistical evidence for a difference.

While some cross-sectional studies show an association between schistosomiasis and prevalent HIV-1 infection [13, 23], this association is not universally reported [34, 35]. The association between schistosomiasis and HIV-1 is complex and cross-study comparisons require the consideration of many aspects, including schistosome species (*S. haematobium* versus *S. mansoni*), diagnostic tests used, assessment of genital involvement, and presentation of subgroup analyses e.g. by participant's sex. Our findings, while limited by power, show a point estimate consistent with increased risk of incident HIV-1, but with a wide confidence interval. Recently, two case-control studies nested within longitudinal African cohorts have retrospectively assessed the association between schistosome infection status and HIV-1 seroconversion with conflicting results [16, 36]. A Zambian study showed an increased risk of HIV-1 acquisition in *S. haematobium* antibody positive women (aHR=1.4, $p<0.05$), but not men [16]. Similar to our results, the study from Kenya and Uganda did not show an association between active schistosome

infection and HIV-1 seroconversion, including in subgroup analyses by sex, schistosome species, and infection intensity [36]. Notably, however, neither of these nested case-control studies evaluated genital infection status. FGS may enhance HIV-1 vulnerability, with proposed mechanisms including cervicovaginal barrier dysfunction [37], local recruitment or activation of HIV-1 target cells [3], and *Schistosoma*-related alterations in integrin [38] or co-receptor [39] expression.

Schistosomiasis and FGS are preventable and current WHO control measures recommend praziquantel preventive chemotherapy [40]. However, current control programmes do not universally achieve 75% coverage of school-aged children, representing substantial missed opportunities for prevention [41, 42]. The 2025 AIDS targets place communities at risk for HIV-1 in the centre of societal, system, and service enablers with a call for between sector integration and synergy to advance the HIV-1 response [43]. Programmatic synergy including integrated sexual and reproductive health programmes could leverage and scale-up existing HIV-1 treatment and prevention resources to include FGS screening and treatment programs.

We carried out two ad hoc exploratory analyses. The intensity of schistosome infection, defined by serum CAA concentration, has been strongly correlated with HIV-1 prevalence [13]. Thus, first, we investigated whether *Schistosoma* DNA concentrations might be associated with HIV-1 infection in an analysis of 13 participants with FGS and moderate/high *Schistosoma* DNA concentrations. We found no evidence of an association between FGS and HIV-1 acquisition, albeit with wide confidence intervals. Additionally, participants with moderate to high intensity seminal egg excretion have higher seminal cytokine concentrations than *S. haematobium* negative

participants [44]. Thus secondly, we investigated the association between multiple positive genital PCR specimens as a potential proxy marker of higher FGS burden and HIV-1 seroconversion in 13 women with ≥2 positive genital specimens for *Schistosoma* DNA. We found weak evidence of an association between FGS and HIV-1 acquisition, which was less pronounced in the age-adjusted estimates. These findings are hypothesis generating for the association between FGS cervicovaginal disease burden or *Schistosoma* DNA concentrations and HIV-1.

This study was nested within a large population-based HIV-1 prevention trial and is the first prospective study to document FGS in Zambia, but also had some relevant limitations. Similar to other FGS studies using imperfect available diagnostics, there is the risk of potential diagnostic misclassification, especially in this low-prevalence setting. We defined FGS by PCR positivity based on its semi-quantitative nature and precedent in FGS diagnosis [17, 18, 45]. However, *S. haematobium* eggs in semen from a male sex partner could potentially be detected by PCR of vaginal specimens. We were unable to adjust for potential confounders beyond age and are thus unable to exclude unmeasured and residual confounding. This was related to the low number of HIV-1 seroconversions and FGS cases, which also resulted in a loss of power. Overall, the effect sizes suggest the possibility of a relationship we were not sufficiently powered to detect. While the prevalence of HIV-1 in the study population was high at 17.9%, the two participating communities were enrolled in HPTN 071 (PopART) as intervention sites, potentially reducing the number of HIV-1 seroconversions [25]. The prevalence of urinary *S. haematobium* infection in this study was 5.5% (27/492), lower than anticipated, defined by the World Health Organization as a low prevalence area (<10% *S. haematobium* prevalence) [46]. Indeed, a 2013 survey done by the Zambian Ministry of Health reported a wide range of egg-patent prevalence in Livingstone,

ranging from of 3.3 – 73.3% (median 15.0%, mean 23.3%) in school-aged children, highlighting its focal distribution [47]. In addition, while schistosomiasis is endemic in all of Zambia's 10 provinces [48], and can be found in urban locations , it is generally considered to be a focal disease of rural areas [49]. For all the above reasons, the presented estimates, obtained in a peri-urban setting, are subject to a high degree of imprecision and may not be generalizable to rural communities. Lastly, vaginal and cervical swabs were self-collected by participants, raising the potential for false negative genital swabs. In future work, this could be addressed by measuring β-globin PCR as a positive control to confirm the presence of human DNA [50]. This study was developed based on a conceptual framework which describes a potentially causal relationship between FGS and HIV-1, with FGS as a potentially preventable and modifiable risk factor. In the literature, albeit in cross-sectional studies, there is evidence for biological plausibility [3, 8, 12] and large effect sizes for the association [7] between FGS and HIV-1. One of our study limitations was the temporality of HIV-1 and FGS diagnostics. HIV-1 seroconversion was measured in HPTN 071 (PopART) up to three years prior to participant enrolment in the study and subsequent FGS diagnosis. This sequencing assumes that FGS status and demographic descriptors at the time of genital PCR sampling are similar to those at the time of HPTN 071 (PopART) study entry and/or HIV-1 seroconversion. This assumption is reasonable given that FGS is thought to develop after childhood water exposure [33] and persist into adulthood with chronic genital lesions, often persisting despite treatment with praziquantel [51]. A large, prospective, longitudinal study in areas of higher *S. haematobium* endemicity is needed to evaluate incident HIV-1 infection in women with known *S. haematobium* and FGS status at study baseline. In future work, it will be important to continue to analyse HIV-1 outcomes by both *S. haematobium* infection status and FGS definition (PCR versus clinical findings) to evaluate HIV-1 risk profiles.

In conclusion, this analysis does not show evidence of association between FGS and HIV-1 incidence. The hypothesis generating observations that FGS, and in particular higher FGS cervicovaginal disease burden or *Schistosoma* DNA concentrations, may be associated with HIV-1 acquisition should be investigated in a larger longitudinal study in a high FGS prevalence area to better explore the role of FGS in HIV-1 acquisition.

Footnotes

Conflict of Interest Statement:

The authors report no conflicts of interest

Funding:

Amaya Bustinduy received funding from the Wellcome Trust (Award 205954/Z/17/Z).

Dr E Webb and Professor R Hayes received funding from MRC Grant Reference MR/K012126/1, and Dr SC Francis received salary from MRC Grant Reference MR/N023692/1. These awards are jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 program supported by the European Union. HPTN 071 (PopART) was supported by the National Institute of Allergy and Infectious Diseases (NIAID) under Cooperative Agreements UM1-AI068619, UM1-AI068617, and UM1-AI068613, with funding from the U.S. President's Emergency Plan for AIDS Relief (PEPFAR); the International Initiative for Impact Evaluation with support from the Bill and Melinda Gates Foundation; the NIAID, the National Institute on Drug Abuse, and the National Institute of Mental Health, all part of the National Institutes of Health. Professor Eyrun Kjetland was supported by South-Eastern Regional Health Authority, Norway project #2016055.

Acknowledgements:

We wish to acknowledge the study participants, without whom this work would not be possible. We would like to recognize the work performed by the BILHIV supervisor Namakau Chola, and the BILHIV community workers Ethel Mwansa, Mwiingana Lukonga, Ruth Mwanza, Mervis

Kantukaleza, and Judith Lungu. We also acknowledge tremendous support in Livingstone from Clement Mwakamui (Zambart) and Tobias Mweene (Zambart). We gratefully acknowledge Eric A.T. Brienen (LUMC) for performing the genital PCR analysis and Claudia J. de Dood (LUMC) and Pytsje T. Hoekstra (LUMC) for performing the CAA analysis.

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REFERENCES

1. UNAIDS. No more neglect — Female genital schistosomiasis and HIV — Integrating sexual and reproductive health interventions to improve women's lives, 2019. Available at: https://www.unaids.org/en/resources/documents/2019/female_genital_schistosomiasis_and_hiv Accessed April 30, 2021.
2. Odegaard JI, Hsieh MH. Immune responses to Schistosoma haematobium infection. Parasite Immunol **2014**; 36:428-38.
3. Sturt AS, Webb EL, Francis SC, Hayes RJ, Bustinduy AL. Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition. Acta Trop **2020**; 209:105524.
4. Poggensee G, Kiwelu I, Weger V, et al. Female genital schistosomiasis of the lower genital tract: prevalence and disease-associated morbidity in northern Tanzania. The Journal of infectious diseases **2000**; 181:1210-3.
5. Kjetland EF, Ndhlovu PD, Mduluza T, et al. Simple clinical manifestations of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2005**; 72:311-9.
6. Ndeffo Mbah ML, Poolman EM, Drain PK, Coffee MP, van der Werf MJ, Galvani AP. HIV and Schistosoma haematobium prevalences correlate in sub-Saharan Africa. Trop Med Int Health **2013**; 18:1174-9.
7. Kjetland EF, Ndhlovu PD, Gomo E, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS **2006**; 20:593-600.
8. Jourdan PM, Holmen SD, Gundersen SG, Roald B, Kjetland EF. HIV target cells in Schistosoma haematobium-infected female genital mucosa. Am J Trop Med Hyg **2011**; 85:1060-4.
9. UNAIDS. UNAIDS Global AIDS Update, Communities at the Centre 2019, available at: https://www.unaids.org/en/20190716_GR2019_communities [accessed April 31, 2021].
10. Birdthistle I, Tanton C, Tomita A, et al. Recent levels and trends in HIV incidence rates among adolescent girls and young women in ten high-prevalence African countries: a systematic review and meta-analysis. The Lancet Global health **2019**; 7:e1521-e40.
11. Patel P, Rose CE, Kjetland EF, et al. Association of schistosomiasis and HIV infections: A systematic review and meta-analysis. Int J Infect Dis **2021**; 102:544-53.
12. Jourdan PM, Roald B, Poggensee G, Gundersen SG, Kjetland EF. Increased vascularity in cervicovaginal mucosa with Schistosoma haematobium infection. PLoS Negl Trop Dis **2011**; 5:e1170.
13. Downs JA, van Dam GJ, Changalucha JM, et al. Association of Schistosomiasis and HIV infection in Tanzania. Am J Trop Med Hyg **2012**; 87:868-73.
14. Ndhlovu PD, Mduluza T, Kjetland EF, et al. Prevalence of urinary schistosomiasis and HIV in females living in a rural community of Zimbabwe: does age matter? Trans R Soc Trop Med Hyg **2007**; 101:433-8.
15. N'Zoukoudi-N'Doundou MY, Dirat I, Akouala JJ, Penchenier L, Makuwa M, Rey JL. [Bilharziasis and human immunodeficiency virus infection in Congo]. Med Trop (Mars) **1995**; 55:249-51.
16. Wall KM, Kilembe W, Vwalika B, et al. Schistosomiasis is associated with incident HIV transmission and death in Zambia. PLoS Negl Trop Dis **2018**; 12:e0006902.

17. Kjetland EF, Hove RJ, Gomo E, et al. Schistosomiasis PCR in vaginal lavage as an indicator of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2009**; 81:1050-5.
18. Sturt AS, Webb EL, Phiri CR, et al. Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. PLoS Negl Trop Dis **2020**; 14:e0008337.
19. Leutscher P, Ravaoalimalala VE, Raharisolo C, et al. Clinical findings in female genital schistosomiasis in Madagascar. Trop Med Int Health **1998**; 3:327-32.
20. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, et al. Gynecological manifestations, histopathological findings, and schistosoma-specific polymerase chain reaction results among women with Schistosoma haematobium infection: a cross-sectional study in Madagascar. The Journal of infectious diseases **2015**; 212:275-84.
21. Galappaththi-Arachchige HN, Holmen S, Koukounari A, et al. Evaluating diagnostic indicators of urogenital Schistosoma haematobium infection in young women: A cross sectional study in rural South Africa. PloS one **2018**; 13:e0191459.
22. Corstjens P, de Dood CJ, Knopp S, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Schistosoma Infections-Improvement and Use during SCORE. Am J Trop Med Hyg **2020**; 103:50-7 doi:10.4269/ajtmh.19-0819.
23. Downs JA, Mguta C, Kaatano GM, et al. Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region. Am J Trop Med Hyg **2011**; 84:364-9.
24. Wright ED, Chiphangwi J, Hutt MS. Schistosomiasis of the female genital tract. A histopathological study of 176 cases from Malawi. Trans R Soc Trop Med Hyg **1982**; 76:822-9.
25. Hayes RJ, Donnell D, Floyd S, et al. Effect of Universal Testing and Treatment on HIV Incidence - HPTN 071 (PopART). N Engl J Med **2019**; 381:207-18.
26. World Health Organization. Female genital schistosomiasis: a pocket atlas for clinical health-care professionals. World Health Organization. 2015. Available at: http://www.who.int/iris/handle/10665/180863 Accessed April 30, 2021.
27. Zambian Ministry of Health. Guidelines for the Etiological and Clinical Management of Sexually Transmitted Infections in Zambia, 2017.
28. Eshleman SH, Piwowar-Manning E, Wilson EA, et al. Determination of HIV status and identification of incident HIV infections in a large, community-randomized trial: HPTN 071 (PopART). J Int AIDS Soc **2020**; 23:e25452.
29. van Lieshout L, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop **2000**; 77:69-80.
30. 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**; 141:1841-55.
31. Obeng BB, Aryeetey YA, de Dood CJ, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. Annals of tropical medicine and parasitology **2008**; 102:625-33.
32. Pillay P, Taylor M, Zulu SG, et al. Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital

Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. Am J Trop Med Hyg **2014**; 90:546-52.
33. Hegertun IE, Sulheim Gundersen KM, Kleppa E, et al. S. haematobium as a common cause of genital morbidity in girls: a cross-sectional study of children in South Africa. PLoS Negl Trop Dis **2013**; 7:e2104.
34. Kapiga S, Hansen CH, Downs JA, et al. The burden of HIV, syphilis and schistosome infection and associated factors among adults in the fishing communities in northwestern Tanzania. Trop Med Int Health **2021**; 26:204-13.
35. Sanya RE, Muhangi L, Nampijja M, et al. Schistosoma mansoni and HIV infection in a Ugandan population with high HIV and helminth prevalence. Trop Med Int Health **2015**; 20:1201-8.
36. Bochner AF, Baeten JM, Secor WE, et al. Associations between schistosomiasis and HIV-1 acquisition risk in four prospective cohorts: a nested case-control analysis. J Int AIDS Soc **2020**; 23:e25534.
37. Feldmeier H, Krantz I, Poggensee G. Female genital schistosomiasis: a neglected risk factor for the transmission of HIV? Trans R Soc Trop Med Hyg **1995**; 89:237.
38. Yegorov S, Joag V, Galiwango RM, et al. Schistosoma mansoni treatment reduces HIV entry into cervical CD4+ T cells and induces IFN-I pathways. Nat Commun **2019**; 10:2296.
39. Kleppa E, Ramsuran V, Zulu S, et al. Effect of female genital schistosomiasis and anti-schistosomal treatment on monocytes, CD4+ T-cells and CCR5 expression in the female genital tract. PloS one **2014**; 9:e98593.
40. World Health Organization. Schistosomiasis. Control and preventive chemotherapy. Available at https://www.who.int/schistosomiasis/strategy/en/. Accessed April 30, 2021.
41. World Health Organization. Schistosomiasis and soil-transmitted helminthiasis: number of people treated in 2016. Available at: https://www.who.int/neglected_diseases/resources/who_wer9249/en/. Accessed April 30, 2021.
42. World Health Organization. Ending the neglect to attain the Sustainable Development Goals A road map for Neglected Tropical Diseases 2021-2030. Available at: https://www.who.int/publications/i/item/9789240010352. Accessed April 30, 2021.
43. UNAIDS. Prevailing against pandemics by putting people at the centre - World AIDS Day report 2020. Available at: https://www.unaids.org/en/resources/documents/2020/prevailing-against-pandemics Accessed April 30, 2021.
44. Leutscher PD, Pedersen M, Raharisolo C, et al. Increased prevalence of leukocytes and elevated cytokine levels in semen from Schistosoma haematobium-infected individuals. The Journal of infectious diseases **2005**; 191:1639-47.
45. Pillay P, van Lieshout L, Taylor M, et al. Cervical cytology as a diagnostic tool for female genital schistosomiasis: Correlation to cervical atypia and Schistosoma polymerase chain reaction. CytoJournal **2016**; 13:10.
46. World Health Organization. Preventive chemotherapy in human helminthiasis : coordinated use of anthelminthic drugs in control interventions : a manual for health professionals and programme managers. 2006. Available at: https://apps.who.int/iris/bitstream/handle/10665/43545/9241547103_eng.pdf;jsessionid=564247BFBA426C9B5D72B1048671034E?sequence=1 Accessed April 30, 2021.

47. Zambian Ministry of Health. Zambia's master plan towards the elimination of Neglected Tropical Diseases, 2015 - 2020. In: Ministry of Community Development, p. 1-188.
48. Kalinda C, Chimbari MJ, Mukaratirwa S. Schistosomiasis in Zambia: a systematic review of past and present experiences. Infect Dis Poverty **2018**; 7:41.
49. Knopp S, Person B, Ame SM, et al. Elimination of schistosomiasis transmission in Zanzibar: baseline findings before the onset of a randomized intervention trial. PLoS Negl Trop Dis **2013**; 7:e2474.
50. Polman NJ, Ebisch RMF, Heideman DAM, et al. Performance of human papillomavirus testing on self-collected versus clinician-collected samples for the detection of cervical intraepithelial neoplasia of grade 2 or worse: a randomised, paired screen-positive, non-inferiority trial. Lancet Oncol **2019**; 20:229-38.
51. Downs JA, Kabangila R, Verweij JJ, et al. Detectable urogenital schistosome DNA and cervical abnormalities 6 months after single-dose praziquantel in women with Schistosoma haematobium infection. Trop Med Int Health **2013**; 18:1090-6.

Table 7.1 – HIV-1 seroconversion rates by baseline BILHIV study characteristic in 492 Zambian women

Socio-behavioural Characteristics		No. (%)	Events	Rate of HIV Seroconversion per 1000 p/y	Crude IRR (95% CI)	p-value
Age in years	18-24	289 (58.7)	15	23.6 (14.2 – 39.2)	Reference	0.06
	25-31	203 (41.3)	5	9.5 (3.9 – 22.7)	0.40 (0.15 – 1.10)	
Marital status	Single	219 (44.5)	12	23.0 (13.1 – 40.5)	Reference	0.35
	Married or Cohabitating	255 (51.8)	7	11.8 (5.6 – 24.7)	0.51 (0.20 – 1.30)	
	Widowed, Divorced, or Separated	18 (3.7)	1	21.2 (3.0 – 150.6)	0.92 (0.12 – 7.10)	
Education (highest level)	None or Any Primary School	137 (27.9)	5	15.7 (6.5 – 37.6)	Reference	0.57
	Any Secondary School	297 (60.4)	11	15.5 (8.6 – 28.0)	0.99 (0.34 – 2.85)	
	Trade, Degree or higher	58 (11.8)	4	29.4 (11.0 – 78.4)	1.88 (0.50 – 7.00)	
District	Community A	260 (52.9)	11	15.5 (8.6 – 28.0)	Reference	0.60
	Community B	232 (47.2)	9	19.8 (10.3 – 38.0)	1.27 (0.53 – 3.07)	
Household members	1-3	141 (28.7)	12	38.4 (21.8 – 67.6)	Reference	0.007$^{\phi}$
	4-5	201 (40.9)	4	8.2 (3.1 – 21.7)	0.21 (0.07 – 0.66)	
	6+	150 (30.5)	4	11.1 (4.2 – 29.5)	0.29 (0.09 – 0.90)	
Employment status	Not Working	327 (66.5)	13	17.1 (10.0 – 29.5)	Reference	0.99
	Working	165 (33.5)	7	17.3 (8.2 – 36.2)	1.01 (0.40 – 2.52)	
Sexual behaviour characteristics						
Age at sexual debut (years)	8-16	197 (40.0)	9	20.6 (10.7 – 39.5)	Reference	0.79
	17-19	220 (44.7)	8	14.9 (7.5 – 29.9)	0.73 (0.28 – 1.88)	
	20-24	75 (15.2)	3	15.7 (5.1 – 48.8)	0.77 (0.21 – 2.83)	

Lifetime sexual partners	1	149 (30.3)	2	5.7 (1.4 – 22.9)	Reference	0.01[ϕ]
	2	134 (27.2)	5	15.9 (6.6 – 38.3)	2.78 (0.54 – 14.34)	
	3	103 (20.9)	5	18.7 (7.8 – 45.0)	3.27 (0.63 – 16.85)	
	4+	106 (21.5)	8	34.1 (17.1 – 68.2)	5.95 (1.26 – 28.02)	
Currently sexually active[**,†]	No	63 (12.9)	3	20.1 (6.5 – 62.4)	Reference	0.78
	Yes	427 (87.1)	17	16.9 (10.5 – 27.1)	0.84 (0.25 – 2.86)	
STI history[††]	No	466 (94.9)	16	14.4 (8.8 – 23.5)	Reference	0.009
	Yes	25 (5.1)	4	82.8 (31.1 – 220.5)	5.76 (1.92 – 17.22)	
Condom use with last sex[†††]	No	367 (75.8)	12	13.8 (7.9 – 24.3)	Reference	0.11
	Yes	117 (24.2)	8	29.2 (14.6 – 58.3)	2.11 (0.86 – 5.16)	
Contraceptive Use						
Condoms	No	407 (82.7)	14	14.8 (8.8 – 25.0)	Reference	0.23
	Yes	85 (17.3)	6	27.5 (12.4 – 61.2)	1.86 (0.71 – 4.83)	
OCP	No	440 (89.4)	18	17.3 (10.9 – 27.4)	Reference	0.96
	Yes	52 (10.6)	2	16.6 (4.1 – 66.3)	0.96 (0.22 – 4.14)	
Injectable	No	225 (45.7)	14	22.5 (13.4 – 38.1)	Reference	0.13
	Yes	267 (54.3)	6	11.1 (5.0 – 24.6)	0.49 (0.19 – 1.28)	
Implant	No	466 (94.7)	18	16.3 (10.3 – 25.9)	Reference	0.37
	Yes	26 (5.3)	2	34.0 (8.5 – 135.8)	2.10 (0.48 – 8.99)	

[ϕ] test for trend p-value
[**]Any sexual activity in the last 6 months
[†]Participants who responded with "no answer" (n=2) are not shown in the table (HIV seroconversions =0)
[††] STI history was self-reported, participants who responded with "no answer" (n=1) are not shown (HIV seroconversions =0)
[†††]Participants who responded with "no answer" (n=8) are not shown in the table (HIV seroconversions =0)

Table 7.2 – HIV-1 incidence by FGS status and schistosomiasis-related factors

Category		N (%)	Incident HIV Cases	Total PY	Rate per 1000 PY (95% CI)	IRR (95% CI)	p-value	aRR*	p-value
FGS Negative**		218 (53.2)	6	532.0	11.3 (5.1 – 25.1)	Reference	0.26#	Reference	0.33#
Probable/possible FGS		166 (40.5)	7	372.2	18.8 (9.0 – 39.5)	1.67 (0.48 – 6.01)		1.73 (0.50 – 6.22)	
FGS		26 (6.3)	2	64.5	31.0 (7.8 – 123.9)	2.75 (0.27 – 15.36)		2.16 (0.21 – 12.30)	
Exploratory Analysis of Participants with FGS									
FGS Negative		218	6	532.0	11.3 (5.1 – 25.1)	Reference	0.09	Reference	0.13
FGS and 2-3 PCR Positive##		13	2	24.9	80.4 (20.1 – 321.7)	7.13 (0.70 – 39.89)		6.02 (0.58 – 34.96)	
FGS Negative		218	6	532.0	11.3 (5.1 – 25.1)	Reference	0.15	Reference	0.19
FGS and PCR Ct<35 †		13	2	32.6	61.31 (15.33 – 245.14)	5.44 (0.54 – 30.40)		4.73 (0.46 – 27.05)	
Schistosomiasis-related factors††									
Urine Microscopy	Negative	465 (94.5)	18	1,102.4	16.3 (10.3 – 25.9)	Reference	0.40	Reference	0.47
	Positive	27 (5.5)	2	61.6	32.5 (8.1 – 129.9)	1.98 (0.46 – 8.58)		1.78 (0.41 – 7.71)	
Urine CAA‡	Not detectable	416 (84.9)	16	993.5	16.1 (9.9 – 26.3)	Reference	0.86	Reference	0.78
	Detectable	74 (15.1)	3	166.6	18.0 (5.8 – 55.8)	1.12 (0.33 – 3.84)		1.19 (0.35 – 4.10)	
Active Infection‡, ‡‡	Not Present	413 (84.3)	16	985.0	16.2 (10.0 – 26.5)	Reference	0.93	Reference	0.85
	Present	77 (15.7)	3	175.1	17.1 (5.5 – 53.1)	1.05 (0.31 – 3.62)		1.13 (0.33 – 3.88)	

[**]82 participants who were negative on all diagnostic tests but missing portable colposcopy images were excluded from this analysis
[*]Adjusted for age
[#]Test for trend p-value (RR per unit of the exposure variable [FGS *Negative*, *Probable/Possible* FGS and FGS] treated as a continuous variable)
[##]n=13 excluded (one genital PCR specimen positive)
[†]n=13 excluded (Ct>35)
[††] n=492, unless otherwise specified
[‡]n=490, 2 vials arrived at the laboratory empty, HIV-1 seroconversion occurred in (n=1) of these participants
[‡‡]defined as detectable urine CAA or positive urine microscopy

Abbreviations: aRR – adjusted rate ratio, CAA – circulating anodic antigen, Ct – cycle threshold, FGS – female genital schistosomiasis, IRR – incidence rate ratio, PCR – polymerase chain reaction, PY – person-years

Figure 7.1 – Female Genital Schistosomiasis categories and Venn diagram illustrating results by diagnostic test type

A

FGS	***Probable* FGS**	***Possible* FGS**	**FGS Negative**
Genital PCR positive for *Schistosoma* DNA	One urine test positive: Urine CAA or Urine microscopy **AND** One of the following colposcopy findings: Homogenous yellow sandy patch, Grainy sandy patch, Rubbery papule, Pathologic blood vessels	One urine test positive: Urine CAA or Urine microscopy **OR** One of the following colposcopy findings: Homogenous yellow sandy patch, Grainy sandy patch, Rubbery papule, Pathologic blood vessels	No diagnostic tests positive

B

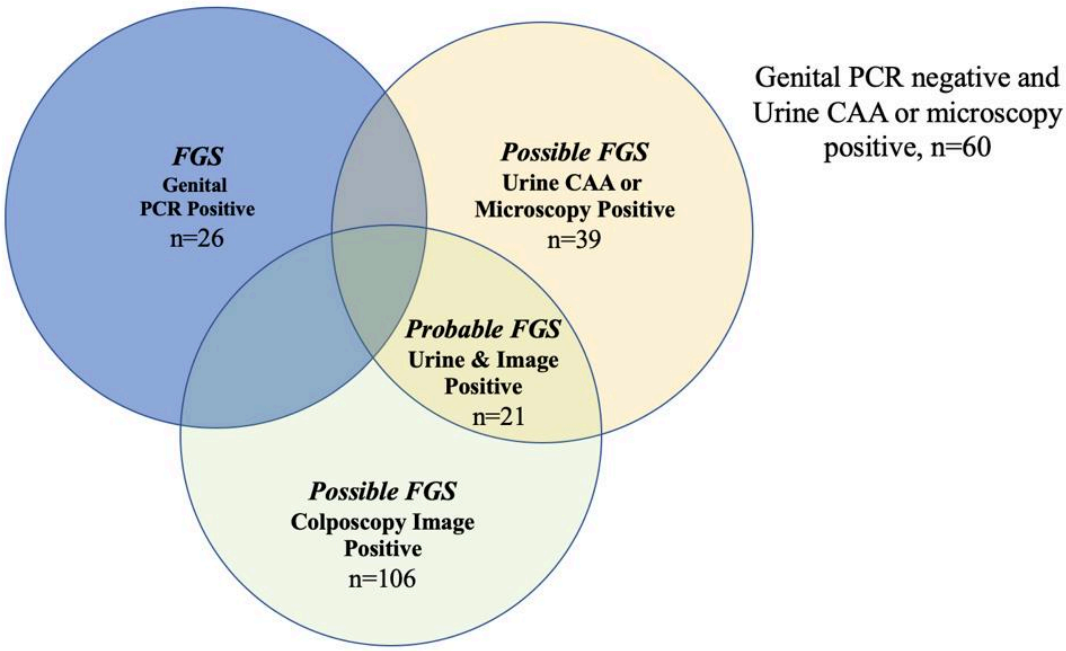


Figure 7.1 Legend:

A. Female Genital Schistosomiasis diagnostic categories

B. Participants in the diagnostic categories by test result

Participants within the FGS and Probable/Possible FGS categories do not overlap.

Figure 7.2 – Study flow diagram

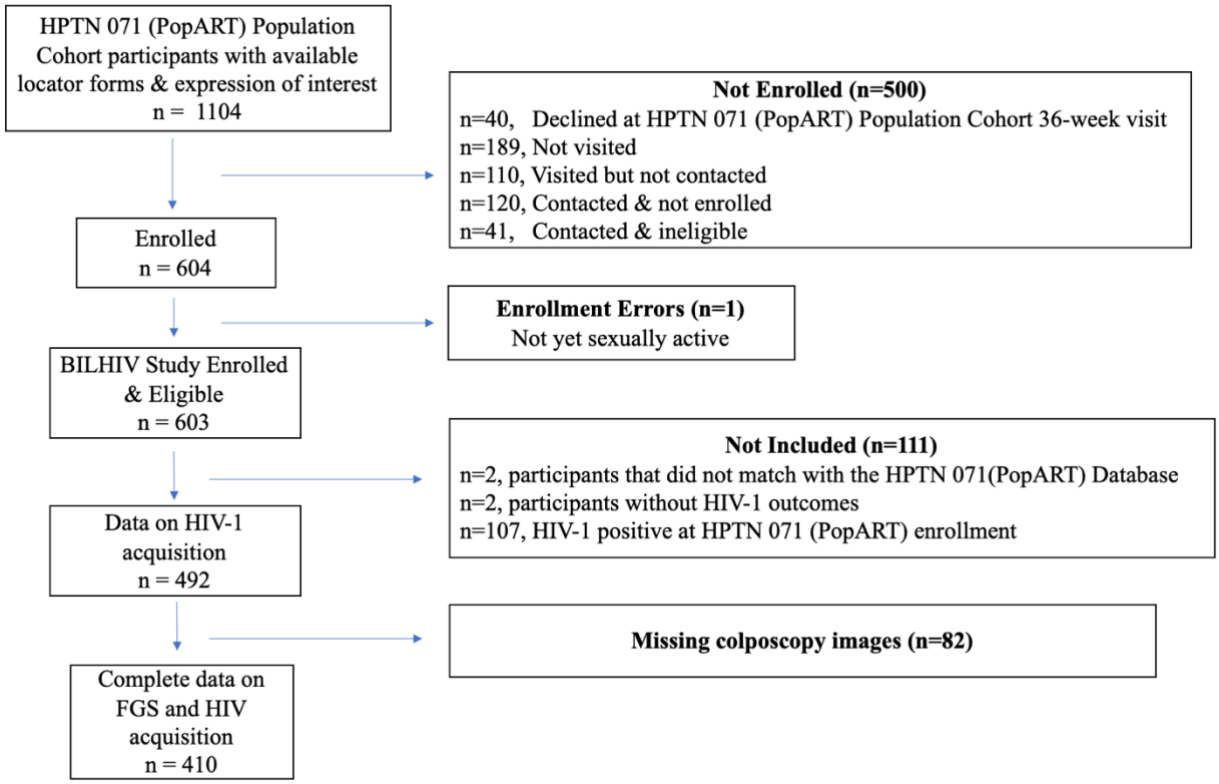


Figure 7.2 Legend:

Not visited (n=189)– the participant was not visited before the study closed for enrolment

Visited but not contacted (n=110)– a visit was made to the study household, but the participant could not be located (70), had relocated (39), or died (1)

Contacted & not immediately enrolled (n=120)– **visited but not recruited** (42), out of town (18), declined to participate (60)

Contacted & ineligible (n=41)– virgin (16), pregnant (17), over 31 (8)

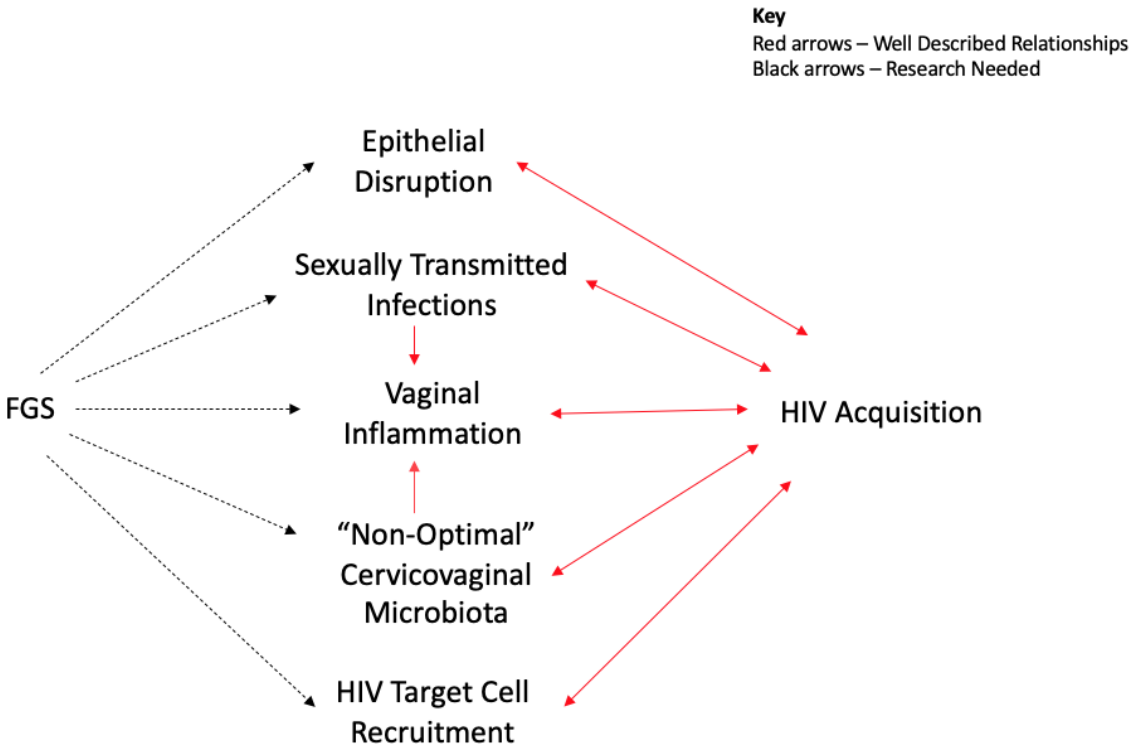
Chapter 8: Discussion and conclusions

8.1 Preface

FGS is a preventable, albeit neglected, tropical gynaecologic condition. Women in endemic countries lack access to adequate diagnostics, curative treatment, and effective prevention [2]. Many even struggle to access adequate water, sanitation and hygiene measures [122]. To develop improved treatment, prevention, and diagnostic efforts, the scientific community needs to better understand FGS pathophysiology and epidemiology. This thesis provides the foundation to leverage additional clinical and laboratory research needed to make concepts translatable to the patient experience.

This chapter outlines the main findings and interpretation of the original research papers, strengths and limitations of the methods, generalisability, and outlines implications for policy and further research. This PhD thesis is comprised of a narrative review and three original research papers. The overall aim of this PhD thesis was to describe the cervicovaginal microbiota (including STIs) and the cervicovaginal immune environment in Zambian women with and without "PCR-defined FGS" and to explore the association of "PCR-defined FGS" with HIV-1 incidence. There is a well-described association between both STIs and "non-optimal" cervicovaginal microbiota and HIV-1 acquisition [100, 123]. Thus, this PhD thesis tests the hypothesis that "PCR-defined FGS" would be associated with STIs and the cervicovaginal microbiota in their "non-optimal" state and that "PCR-defined FGS" would be associated with incident HIV-1 infection. This PhD thesis also proposed that "PCR-defined FGS" might act as a risk factor for HIV-1 acquisition by promoting elevated concentrations of chemokines associated with HIV-1 acquisition and contributing to a change in cervicovaginal cytokine and chemokine concentrations (Figure 8.1).

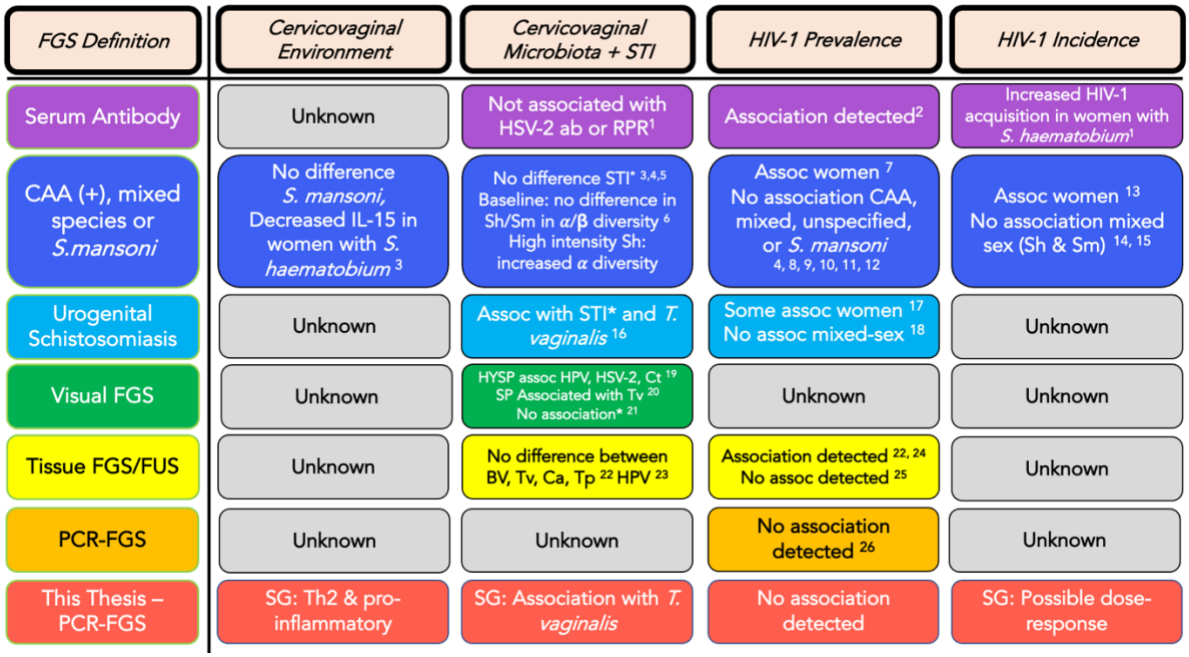
Figure 8.1 – Conceptual pathway describing the potential association of FGS with epithelial disruption, STI, vaginal inflammation, "non-optimal" cervicovaginal microbiota, and HIV-1 target cell recruitment *



**adapted from [14]*

As outlined in *Chapter 1*, FGS studies are challenging to contextualize and compare, due to heterogeneity in study methodologies and FGS definitions. Much research has been performed in participants with "visual FGS" or "urogenital schistosomiasis". This PhD thesis adds to the current FGS literature by using PCR, a semi-quantitative method, to define FGS. Data regarding this emerging standard in FGS research, including work contributed in this PhD thesis, is contextualized with what is currently known regarding other schistosomiasis categories, including "urogenital schistosomiasis", "visual FGS", "tissue FGS", and "unspecified/mixed/*S. mansoni"* infections. Data contributed by this PhD thesis are shown in red in Figure 8.2.

Figure 8.2 – *Schistosoma* infection categories and supporting literature describing the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence, including work from this PhD thesis



Abbreviations: Ab – antibody, Assoc – association, Ca – Candida albicans, CAA – circulating anodic antigen, Ct – *Chlamydia trachomatis*, BV – bacterial vaginosis, diff – difference, HPV – human papillomavirus, HSV-2 – herpes simplex virus-2, HYSP – homogeneous yellow sandy patch, IL – interleukin, FGS – female genital schistosomiasis, RPR – rapid plasma reagin, SG – subgroup, Sh – *S. haematobium*, Sm – *S. mansoni,* SP – sandy patch, STI – sexually transmitted infection, Th2 – T-helper 2, Tp – Treponema pallidum, Tv – Trichomonas vaginalis

8.2 Main findings and Interpretation

8.2.1 Research Paper 2 (Chapter 5): FGS, the cervicovaginal microbiota and STIs – ***Main Findings***

Compared to *negative* FGS women, there was no evidence of a difference in the presence or concentration of cervicovaginal *L. crispatus*, *L. iners*, *A. vaginae*, *G. vaginalis* or *Candida* spp. in participants with "PCR-defined FGS". There was some evidence that *T. vaginalis* was present in a higher proportion of participants with "PCR-defined FGS" (p=0.08) and this association remained after adjusting for age. A secondary analysis combined participants from the "PCR-defined FGS" and *probable* FGS groups. Participants with "PCR-defined FGS"/*probable* FGS likewise had a higher proportions of *T. vaginalis* compared to *negative* FGS participants (p=0.05).

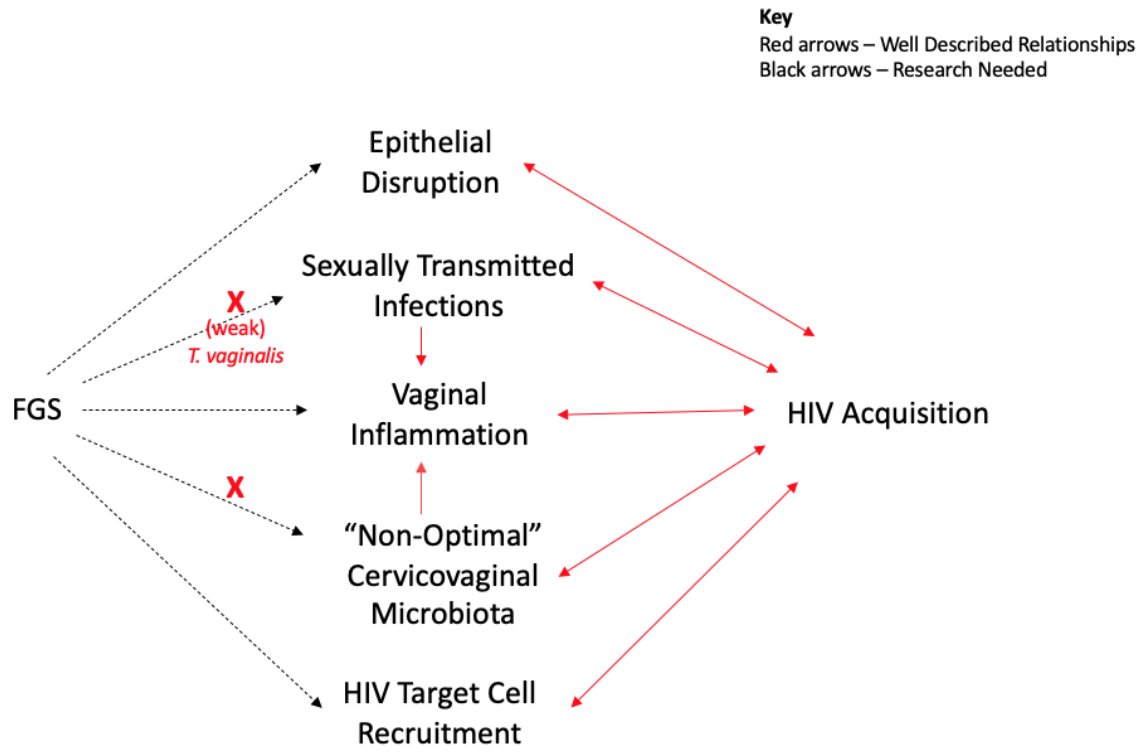
In an exploratory analysis, sixteen participants with a higher burden of "PCR-defined FGS" (≥2 *Schistosoma* PCR positive genital specimens) had an increased prevalence of *T. vaginalis* compared to *negative* FGS participants (p=0.01). In sixteen participants with "PCR-defined FGS" and moderate/high genital *Schistosoma* DNA concentrations, the presence of *T. vaginalis* was higher in the participants with moderate/high *Schistosoma* DNA concentration compared with *negative* FGS participants (p=0.01).

8.2.2 Research Paper 2 (Chapter 5): FGS, the cervicovaginal microbiota and STIs – ***Interpretation***

Molecular characterization of the cervicovaginal microbiota has consistently revealed a variety of bacterial communities that vary in diversity and composition [104, 124]. Low-diversity communities, dominated by lactobacilli have been associated with a lower risk of HIV-1 acquisition compared to higher-diversity anaerobic communities [104]. Additionally, specific cervicovaginal microbiota have been associated with increased risk of HIV-1 acquisition [101]. Thus, at the outset of this work, my hypothesis was that "PCR-defined FGS" would be associated with STIs and the cervicovaginal microbiota in their "non-optimal" state.

The findings presented in *Chapter 5* regarding the cervicovaginal microbiota in women with and without "PCR-defined FGS" harmonise with recently published data from Tanzania. Although using different techniques, 16s rRNA sequencing in Tanzanian women with urinary *S. haematobium* infection showed no difference in cervicovaginal microbiota diversity, compared to uninfected women [59]. Contrary to the original hypothesis, there was no association between the presence and concentration of key species of the cervicovaginal microbiota (*L. crispatus*, *L. iners*, *A. vaginae*, *G. vaginalis*, and *Candida* spp.) with "PCR-defined FGS" (Figure 8.3). This PhD thesis did not evaluate the association of concentrations of cervicovaginal microbiota known to be associated with HIV-1 such as *Parvimonas* species types 1 and 2, *G. asaccharolytica*, *M. hominis*, *Leptotrichia/Sneathia*, *Eggerthella* species type 1, and *Megasphaera* [101].

Figure 8.3 – Conceptual pathway describing the potential association of FGS with epithelial disruption, STI, vaginal inflammation, "non-optimal" cervicovaginal microbiota, and HIV-1 target cell recruitment, with results from Chapter 5



Despite not finding strong evidence of an association between the presence and concentration of cervicovaginal key species, data regarding *T. vaginalis* and FGS co-infection presented in this PhD thesis are thought-provoking. Although there was no association between the STI pathogens *C. trachomatis*, *N. gonorrhoeae*, and *M. genitalium* and "PCR-defined FGS", there was weak evidence that the proportion of participants with *T. vaginalis* was higher in participants with "PCR-defined FGS" compared with *negative* FGS participants (Figure 8.3). The evidence of association between *T. vaginalis* and FGS was strengthened by combining "PCR-defined FGS" and probable FGS participants, due to an increase in power (the point estimate did not change, but the confidence intervals narrowed). Additionally, the association between *T. vaginalis* and FGS was seen across both exploratory analyses of higher FGS burden. This PhD research adds to the growing literature regarding an association between *S. haematobium* infection and *T. vaginalis* [74, 77]. Notably, the association between *S. haematobium* infection and *T. vaginalis* presence has been previously identified across multiple types of *S. haematobium* infection, including "urogenital schistosomiasis" and

"visual FGS" in varied geographical locations with both small and large sample sizes [74, 77].

The association between *T. vaginalis* and FGS raises salient questions regarding the role of the cervicovaginal immunologic environment in FGS pathogenesis. In *Chapter 6*, participants with a higher clinical disease burden of "PCR-defined FGS" had elevated concentrations of the Th2 cytokines (IL-4 and IL-5) in their CVL. A mouse model of *S. haematobium* infection and urinary tract infection suggested that a Th2 environment may be associated with delayed urinary pathogen clearance [125]. If this finding can be extended to the genital tract, future work could evaluate whether "PCR-defined FGS" may be associated with delayed *T. vaginalis* clearance.

Chapter 6 describes a Th2 biased cervicovaginal environment in participants with a higher FGS clinical disease burden. Another potential role of the Th2 immune environment in FGS may involve alternative macrophage activation. Often classically activated macrophages (M1) are selected by pro-inflammatory T-helper (Th) 1 cytokines and alternative macrophage activation (M2) is selected by Th2 cytokines [126]. M2 macrophages are often associated with tissue-repair or fibrosis and can have anti-inflammatory effects [126, 127]. *T. vaginalis* has been implicated in alternative macrophage activation. Macrophage polarization can be also influenced by the local immune environment and schistosome infection, illustrating the complex interplay between the cervicovaginal immune environment and the cervicovaginal microbiota [126, 128].

In a recent meta-analysis, the WHO reports that worldwide prevalence of *T. vaginalis* is 5.3% among women age 15-49, making this parasitic infection the most prevalent non-viral STI [129]. Trichomoniasis is a risk factor for HIV-1 acquisition [130] and the highest global incidence rates have been reported in Africa [129] where increased incident *T. vaginalis* may manifest as a substantial number of new HIV-1 infections in women. Thus, further work is needed to understand how *T. vaginalis* and *S. haematobium* might influence the cervicovaginal environment. As parasites, both *T. vaginalis* and *S. haematobium* require a living host for survival. The unicellular protozoa, *T. vaginalis* is strictly extra-cellular, adhering to cervicovaginal epithelial cells and interacting with host cell galectins to modulate the cervicovaginal immune environment [131-133]. Both *S. haematobium* and *T. vaginalis* interact with the cellular immune system to produce an antibody response, but

asymptomatic infections and re-infection are common to both parasites, possibly suggesting immune evasion or a less effective adaptive immune response [131]. Examining the mechanisms of *T. vaginalis* pathogenesis may provide insights into the association between the parasites. Broadly, *T. vaginalis* influences the local environment first, via attachment to the cervicovaginal epithelium causing disruption of tight junctions and alterations in cellular permeability [134, 135] , second through subversion of innate immunity, via decrease in secretory leukocyte peptidase inhibitor and both direct and soluble-factor lymphotoxic effects on B and T cells [132, 136]; third, through a reduction in epithelia-associated *Lactobacilli [137]* and finally, through haemolysis [138]. The local immune system then counters *T. vaginalis* infection with a pro-inflammatory cytokine and chemokine response. Overlapping synergies between *T. vaginalis* and *S. haematobium* cervicovaginal pathogenesis may begin with, but are not limited to, disruption to the cervicovaginal epithelium, with both infections associated with well-defined clinical manifestations [26, 139]. This breach in the protective cervicovaginal defences provides a plausible mechanism for the HIV-1 vulnerability demonstrated in women with prevalent *T. vaginalis [123]* or *S. haematobium* infection [17].

8.2.3 Research Paper 3 (Chapter 6): FGS and cervicovaginal immune activation – ***Main Findings***

Compared to *negative* FGS women, there was no difference in the concentrations of cytokines and chemokines in participants with "PCR-defined FGS", after adjusting for potential confounders and multiple testing. This includes chemokines (MIP-1a [CCL-3], MIP-1b [CCL-4], IL-8 [CXCL-8], and IP-10 [CXCL-10]) that predicted HIV-1 acquisition risk in a South African study [118]. Using a Principal Components Analysis to reduce the dimensionality of the data, a comparison of the mean scores for the first two Principal Components by "PCR-defined FGS" status, likewise showed no difference between groups.

In exploratory analyses, fifteen participants with a higher "PCR-defined FGS" burden (≥2 *Schistosoma* PCR positive genital specimens) had elevated concentrations of IL-4 and IL-5 compared to *negative* FGS participants, after adjusting for potential confounders and multiple comparisons. Compared to *negative* FGS participants, fifteen participants with "PCR-defined FGS" and moderate/high genital *Schistosoma* DNA concentrations had elevated concentrations of IL-5 and TNF-α, after adjusting for potential confounders and multiple comparisons.

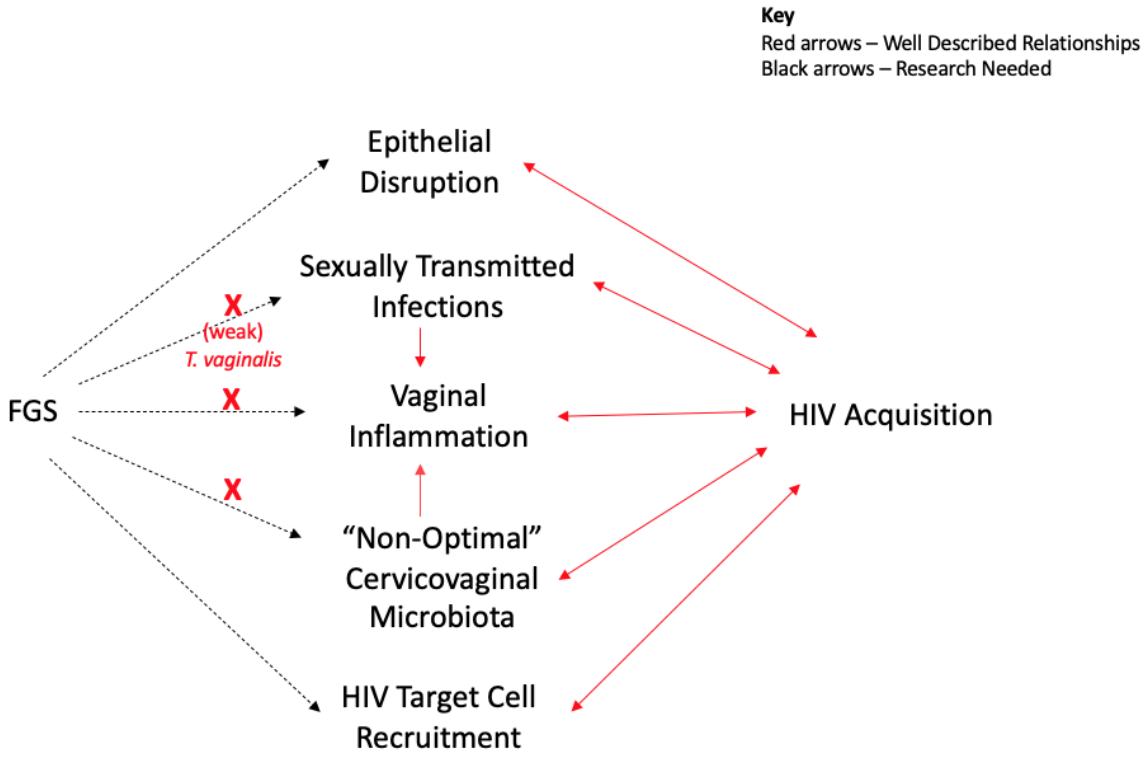
8.2.4 Research Paper 3 (Chapter 6): FGS and cervicovaginal immune activation – ***Interpretation***

The mechanism behind HIV-1 susceptibility in FGS is still largely unknown. Thus, one of the objectives of this PhD thesis was to evaluate whether FGS might be associated with HIV-1 through the promotion of increased concentrations of HIV-1 acquisition associated cytokines. At the outset of this work, my hypothesis was that FGS would act as a risk factor for HIV-1 acquisition by promoting elevated concentrations of chemokines associated with HIV-1 acquisition and contributing to changes in cervicovaginal immune protein concentrations. As presented in *Chapter 6***,** "PCR-defined FGS" and *negative* FGS women had similar expression profiles of chemokines and cytokines, including those that predicted HIV-1 acquisition risk in a South African study (MIP-1a [CCL-3], MIP-1b [CCL-4], IL-8 [CXCL-8], and IP-10 [CXCL-10]) [118]. Thus, "PCR-defined FGS" this thesis suggests that the cytokine and chemokine environment in "PCR-defined FGS" is not likely to increase HIV-1 acquisition risk.

An important finding reported in this thesis is that women with FGS and a high burden of clinical disease displayed a cervicovaginal Th2 environment. Previous work has suggested that HIV-1 strains using the CCR5 co-receptor are primarily responsible for sexual HIV-1 transmission and that the cytokine and chemokine environment can regulate T cell HIV-1 susceptibility [140, 141]. While IL-4 and IL-5 reflect the canonical Th2 cytokines, the presence of mixed Th1 and Th2 immune responses in *S. haematobium* exposure or infection are often reported [142-144]. HIV-1 preferentially replicates in different T cell phenotypes, with a Th17 phenotype supporting CCR5-using HIV-1 replication and a Th2 phenotype supporting only a low to undetectable HIV-1 replication level [140]. Additionally, a Th2 phenotype is associated with replication of CXCR-4 using HIV-1 replication, a co-receptor often associated with chronic HIV-1 infection [140]. While cellular populations were not evaluated in this PhD thesis, overall, the finding of a cervicovaginal Th2-biased immune environment would not support a hypothesis of enhanced HIV-1 susceptibility in FGS [63]. Thus, FGS may instead increase HIV-1 acquisition risk through other mechanisms (Figure 8.4). The HIV-1 vulnerability in *S. mansoni* infection has been seen in women, but not men [56, 57] suggesting that a non-cytokine based response involving the cervicovaginal mucosa may be associated with HIV-1 risk. For example, egg-patent *S. mansoni* infection in women has been associated

higher proportions of mucosal-homing integrin α4β7 expressing cells in the blood [63, 70]. While eggs are primarily deposited in the urogenital tract in *S. haematobium* infection, autopsy studies show that *S. haematobium* eggs can also be found in the colon and small intestine [62], suggesting that further work will need to explore the role of integrin α4β7 in *S. haematobium* infection.

Figure 8.4 – Conceptual pathway describing the potential association of FGS with epithelial disruption, STI, vaginal inflammation, "non-optimal" cervicovaginal microbiota, and HIV-1 target cell recruitment, with results from Chapter 5 and Chapter 6



Another objective of this PhD thesis was to describe the cervicovaginal cytokine and chemokine signature in women with and without FGS, focusing on the Th1 and Th2 immune responses. After adjusting for potential confounders and multiple comparisons, there was no difference in the concentrations of the evaluated chemokines and cytokines between participants who had "PCR-defined FGS" and those who were *negative* FGS. However, in two exploratory analysis there was a difference in Th2 cytokine concentrations (IL-4 and IL-5) between groups when women with a higher clinical burden of infection and in Th2 and pro-inflammatory cytokines (IL-5 and TNF-α, respectively) in those with moderate/high

Schistosoma DNA concentrations were compared with *negative* FGS participants. There was no difference in the canonical Th1 cytokine, IFN-γ between these groups.

This thesis adds to the literature by reporting novel cytokine data in humans with "PCR-defined FGS". Alterations in IL-4 and IL-5 concentrations in women with FGS and a higher clinical disease burden and IL-5 and TNFα concentrations in women with moderate/high genital *Schistosoma* DNA concentrations (reported in *Chapter 6*) harmonise with findings from human and murine research [143-146]. The immunologic response to *Schistosoma* infection is complex and may reflect the interaction of the Th1 and Th2 immune responses [143]. Tissue-lodged *Schistosoma* eggs are accompanied by an influx of T- cells [147]. Indeed, murine *Schistosoma* models report a shift from a Th1 to a Th2 biased immune environment at oviposition and the commencement of egg-patent infection [148]. A tissue-based innate immune response is thought to generate Th2 cells, with IL-4 production supporting granuloma formation, IL-5 production recruiting neutrophils, and IL-13 promoting fibrosis [147]. Conversely, a Th1 and Th17 response may be largely suppressed after oviposition. *Schistosoma* egg antigen as well as Th1 and T regulatory cells induce a regulatory IL-10 response [149]. The Th2 and regulatory responses provide a means for helminths to evade unabated host pro-inflammatory responses [63]. Many such data come from murine models, and while data providing a time course for the cytokine response in human *S. haematobium* infection are lacking, cross-sectional and longitudinal data in human populations have documented that *S. haematobium* infection is associated with altered levels of systemic [144, 150], seminal fluid [151] and cervicovaginal [60] cytokine concentrations.

Notably, Th2 and pro-inflammatory cytokines were present in the CVL of women with a moderate/high concentration of *Schistosoma* DNA. As a marker of schistosomiasis morbidity, TNF-α may be an important mediator of granuloma formation and fibrosis. Morbidity may potentially be correlated with the host's inflammatory response to tissue-lodged ova [146]. While TNF-α expression is not universally reported in *S. haematobium* infection [152], TNF-α production in children with *S. haematobium* infection has been associated with urinary bladder morbidity [146]. Additionally, in vitro models of infection with *T. vaginalis* and cervicovaginal anaerobic bacteria have been associated with cellular tight junction disruption, paracellular permeability, and TNF-α production [135]. The subsequent cervicovaginal barrier disruption may provide access for HIV-1 to underlying target cells. This interesting mechanism

of action brings together work from Chapter 5 and Chapter 6 to explain a potential mechanism that may influence HIV-1 susceptibility. TNF-α is a pro-inflammatory cytokine and is produced by cells from both the innate and adaptive immune system [153]. While TNF-α is produced by Th1 cells, it can also be produced by Th2 cells [154]. Thus, in this thesis TNF-α is referred to as pro-inflammatory rather than a Th1 or Th2 cytokine.

The cytokine work presented in Chapter 6 illustrates the importance of defining and reporting infection intensity in FGS research. The finding that women with "PCR-defined FGS" and moderate/high genital *Schistosoma* DNA concentrations had higher concentrations of Th2 (IL-4) and pro-inflammatory (TNF-α) cytokines than *negative* FGS women, harmonise with research in male genital schistosomiasis. In male genital schistosomiasis, infection intensity has been defined by semen egg count. A study evaluating semen cytokine levels in Madagascan participants (n=116) with male genital schistosomiasis found that men with a moderate/high seminal egg excretion had higher levels of seminal Th2 (IL-4), regulatory (IL-10), Th1 (IFN-γ) and pro-inflammatory (TNF-α) cytokines compared to egg-negative men [151]. Only one study has evaluated cervicovaginal immune proteins in women with schistosomiasis. This study, done in Tanzania, found lower CVL IL-15 concentrations in women with urinary *S. haematobium* infection (n=20, urine microscopy and CAA positive) who had not been evaluated for "PCR-defined FGS". This PhD thesis describes higher concentrations of IL-15 in participants in both exploratory analyses of infection intensity, but there was not strong evidence of a difference between FGS and *negative* FGS groups after adjusting for multiple testing.

The finding that both Th2 and pro-inflammatory cytokines in participants with FGS and a high burden of clinical disease and moderate/high *Schistosoma* DNA concentrations is intriguing and further work will be needed to make these immunologic findings relevant to participants. In the future, immunologic markers of "PCR-defined FGS" could impact FGS diagnostic pathways, may assist in evaluating therapeutics, or could function as markers of treatment success.

8.2.5 Research Paper 4 (Chapter 7): FGS and HIV-1 incidence – ***Main Findings***

Incident HIV-1 infections were observed in 20 (4.1%) of 492 women, who provided 1,164 person-years of follow-up. The overall rate of HIV-1 seroconversions per 1,000 person-years was 17.2 (95% CI 11.1 – 26.6). In women with "PCR-defined FGS", the HIV-1 seroconversion rate was 31.0 [7.8 – 123.9) per 1,000 person-years. This rate of HIV-1 seroconversion was higher than among *negative* FGS participants, albeit without statistical evidence of a difference in both crude and adjusted analyses (crude RR 2.75 [0.27 – 15.36, p=0.26; aRR 2.16 [0.21 – 12.30), p=0.33).

In exploratory analyses, thirteen participants with a higher "PCR-defined FGS" burden (≥2 *Schistosoma* PCR positive genital specimens) acquired HIV-1 with an incidence rate ratio of 6.02 (0.58 – 34.96, p=0.13), compared to *negative* FGS participants, after adjusting for age. Compared to *negative* FGS participants, thirteen participants with "PCR-defined FGS" and moderate/high genital *Schistosoma* DNA concentrations acquired HIV-1 with an incidence rate ratio 4.73 (0.46 – 27.05), p=0.19).

8.2.6 Research Paper 4 (Chapter 7): FGS and HIV-1 Incidence – ***Interpretation***

At the outset of this work, my hypothesis was that "PCR-defined FGS" would be associated with incident HIV-1 infection. The work presented in *Chapter 7* did not show an association between "PCR-defined FGS" and incident HIV-1. In the exploratory analyses of moderate/high *Schistosoma* DNA concentrations, there was no evidence of a difference in HIV-1 seroconversion when comparing participants with a moderate/high *Schistosoma* DNA concentration and *negative* FGS participants. In comparing participants with a higher clinical FGS burden, there was weak evidence of an association with HIV-1 seroconversion, which was less pronounced in the age-adjusted estimates.

Due to low power in the work described in *Chapter 7*, there may have been a difference in HIV-1 seroconversion between "PCR-defined FGS" and *negative* FGS groups that we were unable to detect. The low power makes it challenging to rule out an association between FGS and HIV-1. However, one possible hypothesis is that there may not be an association between FGS and incident HIV-1 and previous work showing an association between "tissue FGS" and HIV-1 [17] may have been influenced by residual or unmeasured confounding. As described in *Chapter 7*, the numbers of HIV-1 seroconversions and the overall numbers of

participants with FGS in both the primary and exploratory analyses were small. Thus, the power to detect a difference between "PCR-defined FGS" and *negative* FGS groups was limited. However, as discussed in *Chapter 1*, the literature on incident HIV-1 in schistosome infection is conflicting, with an increased risk of HIV-1 acquisition in Zambian *S. haematobium* antibody positive women, but not men [47] and no association between active schistosome infection and HIV-1 seroconversion in a study from Kenya and Uganda, despite sub-analyses by sex, schistosome species, and infection intensity [45]. However, neither of these important studies evaluated for genital involvement [45, 47].

In favour of a causal association between "PCR-defined FGS" and prevalent or incident HIV-1, the work presented in *Chapter 7* identifies a possible dose-response relationship between FGS and HIV-1 acquisition and also shows strong effect sizes for this association, albeit with wide confidence intervals [155]. Although the literature on the association between prevalent HIV-1 and FGS are conflicting [83], studies that do show an association between FGS and HIV-1 show a moderate [82] or strong effect size [17]. Also supportive of a potentially causal relationship between FGS and HIV-1 is the hypothesis for biological plausibility. As described in *Chapter 1* and *Chapter 2*, the biological plausibility for an association between FGS and HIV-1 includes FGS characteristics such as mechanical FGS-related cervicovaginal barrier dysfunction [15], the vascular and target cell composition of the granuloma environment [76, 78], enhanced CD4+ T cell trafficking to genital sites through integrin α4β7 expression [70], and potentially alterations in systemic co-receptor expression [77].

8.3 Strengths

This work is the first to describe the cervicovaginal immune environment and the cervicovaginal microbiota in young, sexually active, non-pregnant Zambian women in the context of high HIV-1 prevalence. There are limited data regarding the cervicovaginal immune environment and the microbiota in women with *S. haematobium* infection [59, 60]. This PhD thesis fills a gap in the literature and extends these existing results by evaluating a patient population with "PCR-defined FGS". Previous studies have evaluated HIV-1 acquisition in schistosome infection, however due to the study design, they were not able to evaluate for genital involvement [45, 47]. Thus, a strength of the evaluation of the association of FGS with HIV-1 incidence is the thorough evaluation for "PCR-defined FGS" and "visual FGS" in this study population.

Many studies use 16s rRNA sequencing to evaluate the relative abundance of cervicovaginal microbial communities; however, targeted PCR was chosen for this study. In 16s rRNA approaches, the relative abundance of operational taxonomic units in an individual patient specimen describes the proportion of an operational taxonomic unit of interest within a sample, compared with all other taxa [156]. However, this method does not allow determination of concentrations of individual microbiota, as in PCR. An additional strength of this approach was that it allowed us to define concentrations of the species as interest, as well as identifying a protozoan (*T. vaginalis*) and yeast (*Candida* spp.) that cannot be detected by 16s rRNA sequencing. Another strength is that the immunology work investigated a range of chemokines as well as Th1, Th2, proinflammatory, and regulatory cytokines.

Another strength of this PhD thesis is the strides taken towards filling a number of gaps in the FGS literature. FGS prevalence is likely under documented and we are the first study group to prospectively document FGS in Zambia. The use of biopsy in FGS research has been limited by theoretical concerns regarding HIV-1 vulnerability in the setting of diagnostic biopsy for FGS. Thus, characteristic clinical manifestations of the cervix and vagina have been put forward as a means of FGS diagnosis, but these lesions lack specificity [157]. The use of a semi-quantitative diagnostic with high specificity to define the FGS group enhances the reproducibility of the results [25]. Thus, a strength of this PhD thesis is the use of PCR testing to define the FGS group. Across the three studies presented in *Chapter 5*, *Chapter 6*, and *Chapter 7*, participants are also classified by FGS burden in order to evaluate differences in desired outcomes by clinical burden of disease and moderate/high concentrations of *Schistosoma* DNA. Another strength of the PhD thesis is the ability to classify participants into mutually exclusive categories based on the results of a number of different diagnostics to evaluate for *Schistosoma* infection (urine microscopy and CAA) and FGS (mobile phone colposcopy and *Schistosoma* PCR).

This PhD thesis was based on work performed in the BILHIV study, a cross-sectional study performed among participants previously enrolled in the HPTN 071 (PopART) trial, after the final 36-month visit. The study retention in the BILHIV study was high, with 87.4% (527/603) of enrolled women participating in clinic follow-up. The measurement of HIV-1

incidence in HPTN 071 (PopART) provided a unique opportunity to explore the association of FGS with HIV-1 incidence.

The BILHIV study is one of the largest FGS studies to date, with a total of 603 participants enrolled and evaluated by a comprehensive array of diagnostics: genital PCR, portable colposcopy, CAA and urine microscopy [25]. To maximise resources in the studies of the cervicovaginal microbiota and the cervicovaginal immune environment, all "PCR-defined FGS" cases were frequency matched to a subset of *negative* FGS participants. To reduce the risk of selection bias for the *negative* FGS participants in these studies, a random number generator was used to randomly select *negative* FGS participants matched with "PCR-defined FGS" cases on age group.

8.4 Limitations

Schistosomiasis often affects impoverished areas, lacking in clean water and adequate sanitation [158, 159]. A pre-study survey of school-aged children in the Livingstone area showed a urinary *S. haematobium* prevalence of 20%. Despite this, the prevalence of *S. haematobium* infection by urine microscopy in the BILHIV study was 5.5% (33/603), with 8.2% (26/319) and 2.5% (7/284) prevalence in Community A and B, respectively. Thus, by WHO definitions, Livingstone is a low prevalence area for *S. haematobium* infection [37]. Overall, the prevalence of FGS was 5.7% (30/529). Thus, there were small numbers of FGS cases in both the main and exploratory analyses. The communities enrolled in the BILHIV study had been previously enrolled in intervention arms of the HPTN 071 (PopART) trial [105]. Although HIV-1 prevalence was high (17.9%), the number of HIV-1 seroconversions may have been further reduced due to participants in both Community-A and Community-B receiving trial interventions. Overall, the low prevalence of "PCR-defined FGS" and HIV-1 seroconversion translated into a lack of precision in the HIV-1 incidence study presented in *Chapter 7*. Additionally, in *Chapter 6*, the concentrations of immune proteins measured in CVL were low, despite the use of a protease inhibitor to decrease immune protein degradation. Thus, to reduce the dilution of immune protein concentrations in saline-based CVL in future work, cervicovaginal secretions could be collected in a menstrual cup [160].

The sensitivity of *Schistosoma* PCR is imperfect and varies by the chosen reference standard [25].Currently available diagnostics for schistosomiasis have limited sensitivity, especially in

low-prevalence settings [158]. Although not unique to this study, a limitation when using schistosomiasis diagnostics is the potential risk of diagnostic misclassification. Additionally, women self-collected cervical and vaginal swabs in their homes, raising the possibility of false negative genital swabs. Future work could employ β-globin PCR to assess for the presence of human DNA [161]. This study did not measure prostate specific antigen (PSA) or Y-chromosome to assess for recent penile-vaginal sex [61, 162] and thus cannot preclude that a positive genital specimen may have detected DNA from *Schistosoma* eggs in a male partner's semen.

Another potential limitation is the temporality of HIV-1 and FGS diagnosis. In the HPTN 071 (PopART) trial, HIV-1 may have been diagnosed up to three years prior to the participant's FGS diagnosis. *S. haematobium* infection, and subsequent FGS, likely occurs in childhood and may persist despite treatment with praziquantel [163, 164]. Thus, FGS likely preceded the participant's HIV-1 diagnosis. Ideally, this would be confirmed with the presence of species-specific *Schistosoma* antibodies from HPTN 071 (PopART) banked blood samples.

The BILHIV study was a pilot study comparing the operating characteristics of home-based, self-collected cervical and vaginal swabs with provider performed cervicovaginal lavage [25]. The BILHIV study questionnaire focused on demographics, HIV-1 risk factors, and the acceptability of study procedures and did not enquire regarding menstrual cycle phase, frequency of penile-vaginal sex or intravaginal practices. Additionally, the BILHIV study clinic visit did not assess some behavioural and biological factors that could influence the outcomes of interest in this PhD thesis (cervicovaginal microbiota and the concentration of cytokines and chemokines) such as body weight, PSA or Y-chromosome status, vaginal pH, Nugent score, herpes simplex virus-2, and human papillomavirus status. Additionally, due to the small numbers of outcomes in some analyses, it was not possible to fully adjust for confounding. Thus, we cannot exclude unmeasured or residual confounding. In the studies of the cervicovaginal microbiota and the immunologic environment, we compared multiple key species and 17 cytokines and chemokines. Since these analyses involved multiple statistical comparisons, we adjusted for multiple testing and prioritized results that showed a consistent pattern of association across analyses and species. We also employed two exploratory analyses, the findings of which should be viewed as hypothesis generating.

The cervicovaginal microbiota are dynamic with fluctuations in response to menstrual blood [165], the presence of semen [166], and other behavioural and biological factors. Some cervicovaginal cytokine concentrations may also vary by menstrual cycle phase [107]. The cross-sectional study design limited the ability to characterize the impact of, trends in, and stability of the cervicovaginal microbiota and the immune environment in participants with and without FGS over time. Longitudinal participant follow-up in this cohort would also help minimize inter-individual variability. In this PhD thesis, we measured well-known immune proteins and key species of the cervicovaginal microbiota. However, study results could be expanded by the inclusion of additional specimen types, particularly endocervical cytobrushes for cells for flow cytometry. Due to budgetary and ethical constraints, multi 'omics were not conducted, nor was cellular processing for flow cytometry performed, or biopsy specimens acquired.

8.5 Generalisability

The data presented in this PhD thesis may be generalisable to other sub-Saharan African populations. The BILHIV study population was selected from a representative sample of two communities in Livingstone, Zambia that participated in the HPTN 071 (PopART) trial. A census was performed in each community prior to the HPTN 071 (PopART) trial onset. The household listing was used to select a random sample of households, and each adult in the household was numbered. At the enrolment visit, one adult (age 18 – 44 years) was selected with a random number generator [167]. The BILHIV study population was then selected consecutively from women aged 18 – 31 within the trial population, suggesting a representative sample that should be generalisable to other Zambian communities [25]. We enrolled a population of sexually active, young women from communities with a high HIV-1 prevalence and this population sub-group may be generalisable across sub-Saharan Africa.

Although an estimated 82 million women in sub-Saharan African are living with *Schistosoma* infection [7], FGS prevalence is underreported, with only approximately 10,000 FGS cases described in the published literature [12]. In addition, few studies use PCR-based methods to define FGS [29, 79, 88, 90]. Thus, much progress is needed to ensure the generalisability of the methods employed in this work across sub-Saharan Africa. *Schistosoma* infection is endemic in all of Zambia's 10 provinces [168]. Although often considered a focal disease of rural and impoverished communities [158, 169], schistosomiasis can be found in urban

locations [25, 170]. The BILHIV study was performed in an urban community in Livingstone, Zambia and thus the results may not be generalisable to rural locations and other locations with a higher *S. haematobium* prevalence. In a higher prevalence community, ideally there would have been a higher prevalence of "PCR-defined FGS", allowing greater power for study endpoints.

In the studies of the cervicovaginal microbiota and immune environments presented in *Chapters 5* and *6*, we selected all the participants with "PCR-defined FGS" and probable FGS. The *negative* FGS cases were then frequency matched (on age) to the participants with FGS. Thus, the proportions of demographic and diagnostic variables presented in *Chapter 5* and *6* may not be generalisable to the study population as a whole.

8.6 Implications for further research

This PhD describes higher HIV-1 seroconversion rates in women with "PCR-defined FGS", without statistical evidence of a difference. Evidence of an association between HIV-1 and FGS could fundamentally influence HIV-1 prevention in sub-Saharan Africa. Thus, clarifying the association between HIV-1 and FGS must continue to be a research priority going forward. Evidence of an association between FGS and HIV-1 would likely stimulate the political and societal will to leverage funding needed for expanded praziquantel treatment programmes for FGS-prevention, the integration of FGS into the existing HIV-1 care cascade as well as water, sanitation and hygiene efforts. Clear evidence of an association between FGS and HIV-1 would also likely enhance a funding environment amenable to supporting larger treatment, prevention, and randomised controlled trials testing different treatment interventions. Ideally, further work evaluating HIV-1 incidence in women with "PCR-defined FGS" would be continued with a study design that correctly ascertains the temporality of FGS and HIV-1 seroconversion. In this future work, female HIV-1 negative participants would be evaluated at baseline for "PCR-defined FGS" and "visual FGS", as well as "urogenital schistosomiasis" and followed longitudinally to evaluate HIV-1 seroconversion. Ideally, this work would be performed in a high HIV-1 and high *S. haematobium* prevalence setting. Since the risk profile of both "visual FGS" and "PCR-defined FGS" are relatively unknown, this study would evaluate HIV-1 outcomes in both groups.

Chapter 5 discusses a cross-sectional approach to the immunologic environment in women with "PCR-defined FGS". However, this format leaves open future possibilities to integrate the subject matter of *Chapter 5* and *Chapter 6* in one combined work. Future work could evaluate the impact of praziquantel treatment on cervicovaginal microbiota, cytokines and chemokines in women with FGS followed longitudinally. Additionally, it would be important to evaluate different levels of schistosome infection as the exposure, including "PCR-defined FGS", "visual FGS", and "urogenital schistosomiasis", also including women with high-burden infections. In this prospective cohort Th17 responses could be evaluated in addition to Th1 and Th2 responses both before and after praziquantel treatment. Little is known about the natural history of the human cervicovaginal immune response at different phases of FGS infection (both acute and chronic). Ideally future work could address this knowledge gap. Furthermore, little is known regarding FGS prevalence and intensity in adolescent girls and young women and future efforts in FGS prevention, diagnosis, and treatment could be expanded to include women younger than 18 years of age. The BILHIV study showed that vaginal and cervical self-sampling was acceptable in women 18-31 [171] (Appendix 2), and future work could extend this finding to adolescent girls and young women both before and after their sexual debut.

Currently, there are no data using 16s rRNA sequencing to evaluate the abundance and diversity of the cervicovaginal microbiota in women with "PCR-defined FGS". Future work should evaluate the abundance and diversity of the cervicovaginal microbiota in a prospective cohort both before and after treatment with praziquantel. Additionally, to further evaluate the association of cervicovaginal microbiota with HIV-1 acquisition risk in participants with FGS, future work could evaluate the association of concentrations of cervicovaginal microbiota known to be associated with HIV-1 (*Parvimonas* species types 1 and 2, *G. asaccharolytica*, *M. hominis*, *Leptotrichia/Sneathia*, *Eggerthella* species type 1, and *Megasphaera*) with "PCR-defined FGS" [101].

This PhD describes an association between high burden FGS and the presence of *T. vaginalis*. In addition to aiming to achieve a larger sample size and additional geographically diverse study populations, the next step in evaluating the association between FGS and *T. vaginalis* is to evaluate incident *T. vaginalis* infection in women by differing schistosomiasis definitions ("urogenital schistosomiasis", "visual FGS" and "PCR-defined FGS"). Additional investigations into the pathophysiology of the between-parasite interactions will be important

to define pathogenic mechanisms. Both parasites are known to polarize the macrophage response toward alternative activation [126, 128], which may contribute to *T. vaginalis* persistence. Thus, further research regarding macrophage phenotypes in FGS is indicated. Participants in the sub-studies presented in this PhD thesis were not evaluated for HSV-2 and HPV. A Th2-biased cervicovaginal environment is hypothesized to compromise effective Th1 responses, necessary to control intracellular HPV. Additionally, considering the association between *S. haematobium* and squamous cell carcinoma of the bladder [172], further evaluation between the prevalence and persistence of high and low risk HPV in women with FGS and their cervicovaginal clinical endpoints is also indicated.

The mucosal immune environment contributes substantially to HIV-1 acquisition risk [173]. The immunologic impact of FGS on the female reproductive tract requires additional clarification. In women with "PCR-defined FGS", further work is needed to study human cervicovaginal genes, proteins, and metabolites associated with FGS with the goal of improving FGS prevention, diagnosis, and treatment. Future work needs to focus on establishing links between FGS-related genes, proteins, and metabolites and vaginal immunity and HIV-1 vulnerability. This could be addressed using multi 'omic methods (transcriptomics, proteomics, metabolomics) in women with "PCR-defined FGS" both before and after treatment with praziquantel. Additionally, flow cytometry has not yet been used to characterize the cellular populations present in women with "PCR-defined FGS", especially those cellular populations that are HIV-1 susceptible such as CCR5+ CD4 T-cells and Th17 cells, both before and after treatment with praziquantel [64].

This literature review in *Chapter 1* illustrates the challenges inherent in performing cross-study comparisons in urogenital schistosomiasis and female genital schistosomiasis research. When evaluating schistosomiasis research, multiple components need to be considered including schistosome species, assessment of genital involvement, diagnostics used, reference standard, and analytic strategies (e.g. subgroup analyses by participant sex or intensity). Heterogeneity within these components makes study interpretation and contextualization complex. As evidenced by this PhD thesis, the research community could benefit from consensus definitions that could be applied in research to aid in cross-study comparisons and diagnostic evaluations. Similar classification systems for research purposes are currently in use for challenging to diagnose invasive fungal infections, where a biopsy-based reference standard is not always achievable [174]. Additionally, much of the FGS

literature is cross-sectional, reporting small numbers of affected women, occasionally requiring composite endpoints to assess study outcomes. Further FGS research could be strengthened by evaluating a longitudinal cohort of women, followed over time in communities with varying *S. haematobium* prevalence. Additionally, few "tissue FGS" and "visual FGS" data have been associated with HIV-1, cervicovaginal microbiota, and STI outcomes. These research gaps highlight the opportunity for larger future longitudinal studies.

The WHO definition for FGS includes both the presence of parasite eggs in genital tissue as well as "a characteristic clinical pathology" [24]. However, these findings may represent different ends of a spectrum of *Schistosoma* infection chronicity. Broadly, this spectrum ranges from active infection with genital DNA detection to "visual FGS" with non-viable *S. haematobium* eggs. This PhD thesis employs PCR for *Schistosoma* DNA as a surrogate marker for DNA-containing parasite eggs in genital tissue, however this PCR-based approach misses DNA negative "visual FGS". While the full temporality of viable, egg-producing blood schistosome infection with the development of "visual FGS" manifestations has not yet been described in humans, theoretically, *Schistosoma* egg deposition in genital tissue leads to the development of "visual FGS". However, the relevance of *Schistosoma* DNA positive versus DNA negative "visual FGS" is unknown. Thus, further work could address the DNA status of *S. haematobium* cervicovaginal manifestations and the risk of HIV-1 acquisition.

8.7 Implications for policy

This PhD thesis describes a population of women with "urogenital schistosomiasis" and "PCR-defined FGS". Regardless of whether an association is present between FGS and HIV-1, schistosomiasis and FGS are preventable. Schistosomiasis causes urogenital and intestinal morbidity in all ages [6], with negative cognitive and growth implications for infected children [175, 176]. Preventive chemotherapy for schistosomiasis is currently recommended based on the subject's age and the community schistosomiasis prevalence [37, 158]. Traditionally school age children (age 6-15) and adults from special at-risk groups in moderate-high prevalence areas are targeted for mass drug administration [37, 158], but current control programmes frequently do not meet the target of 75% preventive chemotherapy coverage [177, 178].

Additionally, this PhD thesis illustrates that women aged 18-31, particularly in the sub-group with high intensity infection, experience changes in the cervicovaginal immune environment related to FGS. While this PhD thesis does not provide substantial evidence to change policy, it contributes to the descriptive lexicon regarding the findings and effects of FGS in populations not universally eligible for mass-drug administration [179].

Foundational to this PhD thesis, the BILHIV study illustrates how FGS diagnosis and treatment can be integrated into pre-existing women's health platforms in sub-Saharan Africa, such as cervical cancer screening, to leverage limited human and financial resources [12, 180]. The 2025 joint United Nations Programme on HIV/AIDS (UNAIDS) targets call for between section integration and synergy to advance the HIV-1 response, and the BILHIV study is an example of such integration and synergy [181]. Additionally, the BILHIV community workers were enthusiastic teachers, depicting the how the empowerment of education and awareness can increase advocacy and communication within a community.

8.8 Conclusions

To achieve the United Nations Sustainable Development Goal of "good health and well-being" many countries have focused initiatives around sexual and reproductive health. In achieving this goal, the data presented in this PhD thesis provide a framework from which to approach and leverage funding for future female genital schistosomiasis research. While this thesis represents a modicum of progress, it simultaneously identifies profound deficiencies in the FGS care cascade. Substantial engagement in furthering the FGS research platform is especially needed with regards to FGS epidemiology, treatment, diagnostics, and prevention.

To allow the design and roll-out of effective diagnostic, prevention, and treatment strategies, we need an improved understanding of FGS epidemiology and pathophysiology. Of the utmost importance is resolving the persistent question of whether FGS is associated with HIV-1 acquisition. A definitive answer is urgently needed and will require an adequately powered, longitudinal study in areas of high *S. haematobium* and HIV-1 endemicity. In addition to providing data on the association between FGS and incident HIV-1, longitudinal data will fill critical knowledge gaps regarding FGS epidemiology and the effect of praziquantel treatment.

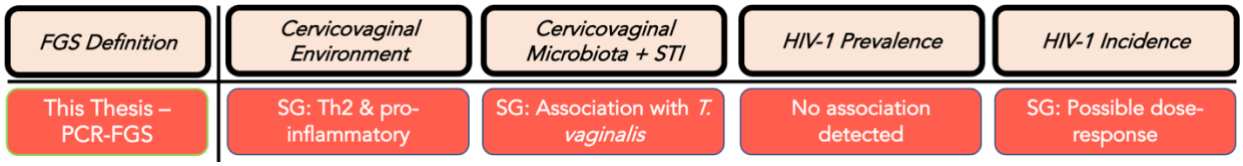
The ASSURED criteria declare that diagnostics for infectious tropical diseases should be affordable, sensitive, specific, user-friendly, rapid, equipment free, and delivered at all levels of the healthcare system [182]. Current FGS diagnostics fall profoundly short of the ASSURED criteria. Thus, substantial effort must be invested in the further development point-of-care ASSURED molecular assays, such as the recombinase polymerase assay (RPA) which has been piloted in urogenital schistosomiasis [183]. The RPA must be further evaluated in FGS, ideally in combination with genital self-sampling [25]. Combining RPA and genital self-sampling for community FGS diagnosis should be investigated in areas of varying *S. haematobium* endemicity with the aim to map, define, and evaluate FGS prevalence and burden. Additionally, with ASSURED diagnostics, FGS diagnosis and management could be seamlessly integrated into pre-existing comprehensive sexual and reproductive health services for cervical cancer, STIs, and HIV-1 [184]. An additional challenge in the field is the absence of a reference standard for FGS research initiatives. Future work must thoughtfully and sensitively address bias around the use of cervicovaginal biopsy in FGS [185] and strive to unite the FGS community around meaningful, reproducible, and consistent diagnostic research endpoints.

In addition to sensitive point-of-care FGS diagnostics, women in *S. haematobium* endemic areas require improved access to curative FGS treatment. Currently, praziquantel is recommended for FGS treatment. Though studies vary by use of biopsy, lesion type, duration of follow-up, and praziquantel dose, a proportion of FGS lesions (ranging from 29 – 100%) are not reversible after a single praziquantel treatment [14, 164]. This highlights both the urgent need for improved FGS treatment modalities and effective prevention strategies in girls and young women prior to the development of clinical FGS manifestations.

This PhD thesis is the first research to describe the immunologic environment and the presence and concentrations of key cervicovaginal key species and STIs among women with "PCR-defined FGS" (Figure 8.5). The cervicovaginal environment is an important determinant of sexual and reproductive health and influences the risk of HIV-1 acquisition [91, 118, 186]. HIV-1 incidence was evaluated in the context of a cross-sectional study of young, sexually active Zambian women with "PCR-defined FGS". The findings in this PhD thesis suggest that the burden of FGS infection, whether defined by *Schistosoma* DNA

concentrations or number of positive clinical specimens, is an important variable to consider in FGS research.

Figure 8.5 – Evidence from this PhD thesis regarding "PCR-defined FGS" and the cervicovaginal environment, the cervicovaginal microbiota, HIV-1 prevalence and HIV-1 incidence



Abbreviations: FGS – female genital schistosomiasis, HIV-1 – human immunodeficiency virus, PCR – polymerase chain reaction, SG – sub-group, STI – sexually transmitted infection, Th2 – T helper 2

Women with moderate to high concentrations of *Schistosoma* DNA and higher clinical burden "PCR-defined FGS" have higher concentrations of pro-inflammatory/Th2 and Th2 cytokines, respectively, present in their cervicovaginal lavage compared to *negative* FGS women. Work presented in this PhD thesis also describes that women in these sub-groups, whether assessed by clinical burden or DNA concentration, were more likely to have cervicovaginal *T. vaginalis* present than *negative* FGS participants. While there was no evidence of a difference in the rates of HIV-1 acquisition between women with and without "PCR-defined FGS", there may be a dose-response relationship between higher burden genital *Schistosoma* DNA concentrations or multiple positive specimens and HIV-1 seroconversion.

This thesis evaluates the cervicovaginal environment in Zambian women with and without FGS and evaluates the association of FGS with HIV-1 incidence. Further work investigating the cervicovaginal environment and HIV-1 risk in FGS will be needed to impact diagnostic, preventative, and therapeutic options for women with FGS. Additionally, prospective studies of the cervicovaginal microbiome, multi 'omics, and flow cytometry in women focusing on epithelial disruption and target cell recruitment in women with FGS will help define and describe the HIV-1 susceptibility in this vulnerable population.

References

1. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet **2014**; 383:2253-64.
2. Hotez PJ, Engels D, Gyapong M, Ducker C, Malecela MN. Female Genital Schistosomiasis. N Engl J Med **2019**; 381:2493-5.
3. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lancet **2006**; 368:1106-18.
4. Nation CS, Da'dara AA, Marchant JK, Skelly PJ. Schistosome migration in the definitive host. PLoS Negl Trop Dis **2020**; 14:e0007951.
5. McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, Zhou XN. Schistosomiasis. Nat Rev Dis Primers **2018**; 4:13.
6. World Health Organization. "Schistosomiasis". Geneva, 2020. Available at: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis Accessed April 30, 2021.
7. Lai YS, Biedermann P, Ekpo UF, et al. Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: a systematic review and geostatistical analysis. Lancet Infect Dis **2015**; 15:927-40.
8. Poggensee G, Kiwelu I, Saria M, Richter J, Krantz I, Feldmeier H. Schistosomiasis of the lower reproductive tract without egg excretion in urine. Am J Trop Med Hyg **1998**; 59:782-3.
9. Szela E, Bachicha J, Miller D, Till M, Wilson JB. Schistosomiasis and cervical cancer in Ghana. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics **1993**; 42:127-30.
10. Kjetland EF, Poggensee G, Helling-Giese G, et al. Female genital schistosomiasis due to Schistosoma haematobium. Clinical and parasitological findings in women in rural Malawi. Acta Trop **1996**; 62:239-55.
11. Renaud G, Devidas A, Develoux M, Lamothe F, Bianchi G. Prevalence of vaginal schistosomiasis caused by Schistosoma haematobium in an endemic village in Niger. Trans R Soc Trop Med Hyg **1989**; 83:797.
12. Engels D, Hotez PJ, Ducker C, et al. Integration of prevention and control measures for female genital schistosomiasis, HIV and cervical cancer. Bull World Health Organ **2020**; 98:615-24.
13. UNAIDS. UN Joint Programme on HIV/AIDS. Global HIV & AIDS statistics — 2020 fact sheet at: https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf Accessed April 30, 2021].
14. Sturt AS, Webb EL, Francis SC, Hayes RJ, Bustinduy AL. Beyond the barrier: Female Genital Schistosomiasis as a potential risk factor for HIV-1 acquisition. Acta Trop **2020**; 209:105524.
15. Feldmeier H, Krantz I, Poggensee G. Female genital schistosomiasis as a risk-factor for the transmission of HIV. Int J STD AIDS **1994**; 5:368-72.
16. Ndeffo Mbah ML, Poolman EM, Drain PK, Coffee MP, van der Werf MJ, Galvani AP. HIV and Schistosoma haematobium prevalences correlate in sub-Saharan Africa. Trop Med Int Health **2013**; 18:1174-9.
17. Downs JA, Mguta C, Kaatano GM, et al. Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region. Am J Trop Med Hyg **2011**; 84:364-9.

18. Ndhlovu PD, Mduluza T, Kjetland EF, et al. Prevalence of urinary schistosomiasis and HIV in females living in a rural community of Zimbabwe: does age matter? Trans R Soc Trop Med Hyg **2007**; 101:433-8.
19. N'Zoukoudi-N'Doundou MY, Dirat I, Akouala JJ, Penchenier L, Makuwa M, Rey JL. [Bilharziasis and human immunodeficiency virus infection in Congo]. Med Trop (Mars) **1995**; 55:249-51.
20. Gelfand M, Ross MD, Blair DM, Weber MC. Distribution and extent of schistosomiasis in female pelvic organs, with special reference to the genital tract, as determined at autopsy. Am J Trop Med Hyg **1971**; 20:846-9.
21. Edington GM, Nwabuebo I, Junaid TA. The pathology of schistosomiasis in Ibadan, Nigeria with special reference to the appendix, brain, pancreas and genital organs. Trans R Soc Trop Med Hyg **1975**; 69:153-6.
22. Poggensee G, Kiwelu I, Weger V, et al. Female genital schistosomiasis of the lower genital tract: prevalence and disease-associated morbidity in northern Tanzania. The Journal of infectious diseases **2000**; 181:1210-3.
23. Helling-Giese G, Sjaastad A, Poggensee G, et al. Female genital schistosomiasis (FGS): relationship between gynecological and histopathological findings. Acta Trop **1996**; 62:257-67.
24. Word Health Organization. Report of an informal working group meeting on urogenital schistosomiasis and HIV transmission. Geneva: World Health Organization; 2009. pp 1-35. Available at: https://www.who.int/schistosomiasis/resources/who_htm_ntd_pct_2010.5/en/ Accessed April 30, 2021.
25. Sturt AS, Webb EL, Phiri CR, et al. Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. PLoS Negl Trop Dis **2020**; 14:e0008337.
26. Kjetland EF, Ndhlovu PD, Mduluza T, et al. Simple clinical manifestations of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2005**; 72:311-9.
27. World Health Organization. Female genital schistosomiasis: a pocket atlas for clinical health-care professionals. World Health Organization. 2015. Available at: http://www.who.int/iris/handle/10665/180863 Accessed April 30, 2021.
28. Kjetland EF, Norseth HM, Taylor M, et al. Classification of the lesions observed in female genital schistosomiasis. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics **2014**; 127:227-8.
29. Kjetland EF, Hove RJ, Gomo E, et al. Schistosomiasis PCR in vaginal lavage as an indicator of genital Schistosoma haematobium infection in rural Zimbabwean women. Am J Trop Med Hyg **2009**; 81:1050-5.
30. Corstjens PLAM, de Dood CJ, Knopp S, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Schistosoma Infections—Improvement and Use during SCORE. The American journal of tropical medicine and hygiene **2020**.
31. 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**; 141:1841-55.
32. de Dood CJ, Hoekstra PT, Mngara J, et al. Refining Diagnosis of Schistosoma haematobium Infections: Antigen and Antibody Detection in Urine. Front Immunol **2018**; 9:2635.

33. Kildemoes AO, Vennervald BJ, Tukahebwa EM, et al. Rapid clearance of Schistosoma mansoni circulating cathodic antigen after treatment shown by urine strip tests in a Ugandan fishing community - Relevance for monitoring treatment efficacy and re-infection. PLoS Negl Trop Dis **2017**; 11:e0006054.
34. de Jonge N, De Caluwe P, Hilberath GW, Krijger FW, Polderman AM, Deelder AM. Circulating anodic antigen levels in serum before and after chemotherapy with praziquantel in schistosomiasis mansoni. Trans R Soc Trop Med Hyg **1989**; 83:368-72.
35. Kremsner PG, Enyong P, Krijger FW, et al. Circulating anodic and cathodic antigen in serum and urine from Schistosoma haematobium-infected Cameroonian children receiving praziquantel: a longitudinal study. Clin Infect Dis **1994**; 18:408-13.
36. Knopp S, Corstjens PL, Koukounari A, et al. Sensitivity and Specificity of a Urine Circulating Anodic Antigen Test for the Diagnosis of Schistosoma haematobium in Low Endemic Settings. PLoS Negl Trop Dis **2015**; 9:e0003752.
37. World Health Organization. Preventive chemotherapy in human helminthiasis : coordinated use of anthelminthic drugs in control interventions : a manual for health professionals and programme managers. 2006. Available at: https://apps.who.int/iris/bitstream/handle/10665/43545/9241547103_eng.pdf;jsessionid=564247BFBA426C9B5D72B1048671034E?sequence=1 Accessed April 30, 2021.
38. Doehring E, Ehrich JH, Vester U, Feldmeier H, Poggensee U, Brodehl J. Proteinuria, hematuria, and leukocyturia in children with mixed urinary and intestinal schistosomiasis. Kidney Int **1985**; 28:520-5.
39. Knopp S, Ame SM, Hattendorf J, et al. Urogenital schistosomiasis elimination in Zanzibar: accuracy of urine filtration and haematuria reagent strips for diagnosing light intensity Schistosoma haematobium infections. Parasites & vectors **2018**; 11:552.
40. Montresor A Crompton David W. T Hall A Bundy D. A. P Savioli L. et al. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level : a guide for managers of control programmes. Geneva: World Health Organization; 1998. Available at: https://apps.who.int/iris/handle/10665/63821. Accessed April 30, 2021.
41. Knopp S, Person B, Ame SM, et al. Evaluation of integrated interventions layered on mass drug administration for urogenital schistosomiasis elimination: a cluster-randomised trial. The Lancet Global health **2019**; 7:e1118-e29.
42. King CH, Bertsch D. Meta-analysis of urine heme dipstick diagnosis of Schistosoma haematobium infection, including low-prevalence and previously-treated populations. PLoS Negl Trop Dis **2013**; 7:e2431.
43. Ochodo EA, Gopalakrishna G, Spek B, et al. Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. Cochrane Database Syst Rev **2015**:CD009579.
44. Krauth SJ, Greter H, Stete K, et al. All that is blood is not schistosomiasis: experiences with reagent strip testing for urogenital schistosomiasis with special consideration to very-low prevalence settings. Parasites & vectors **2015**; 8:584.
45. Bochner AF, Baeten JM, Secor WE, et al. Associations between schistosomiasis and HIV-1 acquisition risk in four prospective cohorts: a nested case-control analysis. J Int AIDS Soc **2020**; 23:e25534.
46. Hinz R, Schwarz NG, Hahn A, Frickmann H. Serological approaches for the diagnosis of schistosomiasis - A review. Mol Cell Probes **2017**; 31:2-21.
47. Wall KM, Kilembe W, Vwalika B, et al. Schistosomiasis is associated with incident HIV transmission and death in Zambia. PLoS Negl Trop Dis **2018**; 12:e0006902.

48. Stabinski L, Reynolds SJ, Ocama P, et al. High prevalence of liver fibrosis associated with HIV infection: a study in rural Rakai, Uganda. Antivir Ther **2011**; 16:405-11.
49. van Lieshout L, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop **2000**; 77:69-80.
50. Fontanet AL, Woldemichael T, Sahlu T, et al. Epidemiology of HIV and Schistosoma mansoni infections among sugar-estate residents in Ethiopia. Annals of tropical medicine and parasitology **2000**; 94:145-55.
51. Kallestrup P, Zinyama R, Gomo E, et al. Schistosomiasis and HIV-1 infection in rural Zimbabwe: implications of coinfection for excretion of eggs. The Journal of infectious diseases **2005**; 191:1311-20.
52. Downs JA, van Dam GJ, Changalucha JM, et al. Association of Schistosomiasis and HIV infection in Tanzania. Am J Trop Med Hyg **2012**; 87:868-73.
53. Mazigo HD, Dunne DW, Wilson S, et al. Co-infection with Schistosoma mansoni and Human Immunodeficiency Virus-1 (HIV-1) among residents of fishing villages of north-western Tanzania. Parasites & vectors **2014**; 7:587.
54. Sanya RE, Muhangi L, Nampijja M, et al. Schistosoma mansoni and HIV infection in a Ugandan population with high HIV and helminth prevalence. Trop Med Int Health **2015**; 20:1201-8.
55. Ssetaala A, Nakiyingi-Miiro J, Asiki G, et al. Schistosoma mansoni and HIV acquisition in fishing communities of Lake Victoria, Uganda: a nested case-control study. Trop Med Int Health **2015**; 20:1190-5.
56. Downs JA, de Dood CJ, Dee HE, et al. Schistosomiasis and Human Immunodeficiency Virus in Men in Tanzania. Am J Trop Med Hyg **2017**; 96:856-62.
57. Downs JA, Dupnik KM, van Dam GJ, et al. Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load at HIV-1 seroconversion: A nested case-control study. PLoS Negl Trop Dis **2017**; 11:e0005968.
58. Kapiga S, Hansen CH, Downs JA, et al. The burden of HIV, syphilis and schistosome infection and associated factors among adults in the fishing communities in northwestern Tanzania. Trop Med Int Health **2021**; 26:204-13.
59. Bullington BW, Lee MH, Mlingi J, et al. Cervicovaginal bacterial communities in reproductive-aged Tanzanian women with Schistosoma mansoni, Schistosoma haematobium, or without schistosome infection. ISME J **2021**.
60. Dupnik KM, Lee MH, Mishra P, et al. Altered Cervical Mucosal Gene Expression and Lower Interleukin 15 Levels in Women With Schistosoma haematobium Infection but Not in Women With Schistosoma mansoni Infection. The Journal of infectious diseases **2019**; 219:1777-85.
61. Yegorov S, Galiwango RM, Good SV, et al. Schistosoma mansoni infection and socio-behavioural predictors of HIV risk: a cross-sectional study in women from Uganda. BMC Infect Dis **2018**; 18:586.
62. Cheever AW, Kamel IA, Elwi AM, Mosimann JE, Danner R. Schistosoma mansoni and S. haematobium infections in Egypt. II. Quantitative parasitological findings at necropsy. Am J Trop Med Hyg **1977**; 26:702-16.
63. Yegorov S, Joag V, Galiwango RM, Good SV, Okech B, Kaul R. Impact of Endemic Infections on HIV Susceptibility in Sub-Saharan Africa. Trop Dis Travel Med Vaccines **2019**; 5:22.

64. Mehta SD, Okal D, Otieno F, et al. Schistosomiasis is associated with rectal mucosal inflammation among Kenyan men who have sex with men. Int J STD AIDS **2021**:956462420985973.
65. Nazli A, Chan O, Dobson-Belaire WN, et al. Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. PLoS Pathog **2010**; 6:e1000852.
66. 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.
67. Poggensee G, Krantz I, Kiwelu I, Diedrich T, Feldmeier H. Presence of Schistosoma mansoni eggs in the cervix uteri of women in Mwanga District, Tanzania. Trans R Soc Trop Med Hyg **2001**; 95:299-300.
68. Kayuni S, Lampiao F, Makaula P, et al. A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa. Parasite Epidemiol Control **2019**; 4:e00077.
69. Gill N, Wlodarska M, Finlay BB. The future of mucosal immunology: studying an integrated system-wide organ. Nat Immunol **2010**; 11:558-60.
70. Yegorov S, Joag V, Galiwango RM, et al. Schistosoma mansoni treatment reduces HIV entry into cervical CD4+ T cells and induces IFN-I pathways. Nat Commun **2019**; 10:2296.
71. Stieh DJ, Matias E, Xu H, et al. Th17 Cells Are Preferentially Infected Very Early after Vaginal Transmission of SIV in Macaques. Cell Host Microbe **2016**; 19:529-40.
72. Prodger JL, Ssemaganda A, Ssetaala A, et al. Schistosoma mansoni Infection in Ugandan Men Is Associated with Increased Abundance and Function of HIV Target Cells in Blood, but Not the Foreskin: A Cross-sectional Study. PLoS Negl Trop Dis **2015**; 9:e0004067.
73. Secor WE, Shah A, Mwinzi PM, Ndenga BA, Watta CO, Karanja DM. Increased density of human immunodeficiency virus type 1 coreceptors CCR5 and CXCR4 on the surfaces of CD4(+) T cells and monocytes of patients with Schistosoma mansoni infection. Infect Immun **2003**; 71:6668-71.
74. Gadoth A, Mvumbi G, Hoff NA, et al. Urogenital Schistosomiasis and Sexually Transmitted Coinfections among Pregnant Women in a Schistosome-Endemic Region of the Democratic Republic of Congo. Am J Trop Med Hyg **2019**; 101:828-36.
75. Dupnik KM, Reust MJ, Vick KM, et al. Gene Expression Differences in Host Response to Schistosoma haematobium Infection. Infect Immun **2019**; 87.
76. Jourdan PM, Holmen SD, Gundersen SG, Roald B, Kjetland EF. HIV target cells in Schistosoma haematobium-infected female genital mucosa. Am J Trop Med Hyg **2011**; 85:1060-4.
77. Kleppa E, Ramsuran V, Zulu S, et al. Effect of female genital schistosomiasis and anti-schistosomal treatment on monocytes, CD4+ T-cells and CCR5 expression in the female genital tract. PloS one **2014**; 9:e98593.
78. Jourdan PM, Roald B, Poggensee G, Gundersen SG, Kjetland EF. Increased vascularity in cervicovaginal mucosa with Schistosoma haematobium infection. PLoS Negl Trop Dis **2011**; 5:e1170.
79. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, et al. Gynecological manifestations, histopathological findings, and schistosoma-specific polymerase chain reaction results among women with Schistosoma haematobium infection: a cross-sectional study in Madagascar. The Journal of infectious diseases **2015**; 212:275-84.
80. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA Trichomonas vaginalis transcription-mediated amplification to wet mount microscopy, culture, and polymerase

chain reaction for diagnosis of trichomoniasis in men and women. Am J Obstet Gynecol **2009**; 200:188 e1-7.
81. Bachmann LH, Hobbs MM, Sena AC, et al. Trichomonas vaginalis genital infections: progress and challenges. Clin Infect Dis **2011**; 53 Suppl 3:S160-72.
82. Kjetland EF, Ndhlovu PD, Gomo E, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS **2006**; 20:593-600.
83. Yirenya-Tawaiah D Amoah C Apea-Kubi K Dade M Annang T Bosompem KM. HIV testing in community-based research: a case study of female genital schistosomiasis and HIV in the Volta Basin of Ghana. J Ghana Sci Assoc. 2009; 11: unpaginated. Available at: https://www.ajol.info//index.php/jgsa/article/view/46997.
84. Leutscher P, Raharisolo C, Pecarrere JL, et al. Schistosoma haematobium induced lesions in the female genital tract in a village in Madagascar. Acta Trop **1997**; 66:27-33.
85. Talaat M, Watts S, Mekheimar S, Farook Ali H, Hamed H. The social context of reproductive health in an Egyptian hamlet: a pilot study to identify female genital schistosomiasis. Soc Sci Med **2004**; 58:515-24.
86. Leutscher P, Ravaoalimalala VE, Raharisolo C, et al. Clinical findings in female genital schistosomiasis in Madagascar. Trop Med Int Health **1998**; 3:327-32.
87. Poggensee G, Sahebali S, Van Marck E, Swai B, Krantz I, Feldmeier H. Diagnosis of genital cervical schistosomiasis: comparison of cytological, histopathological and parasitological examination. Am J Trop Med Hyg **2001**; 65:233-6.
88. Galappaththi-Arachchige HN, Holmen S, Koukounari A, et al. Evaluating diagnostic indicators of urogenital Schistosoma haematobium infection in young women: A cross sectional study in rural South Africa. PloS one **2018**; 13:e0191459.
89. Pillay P, Downs JA, Changalucha JM, et al. Detection of Schistosoma DNA in genital specimens and urine: A comparison between five female African study populations originating from S. haematobium and/or S. mansoni endemic areas. Acta Trop **2020**; 204:105363.
90. Pillay P, van Lieshout L, Taylor M, et al. Cervical cytology as a diagnostic tool for female genital schistosomiasis: Correlation to cervical atypia and Schistosoma polymerase chain reaction. CytoJournal **2016**; 13:10.
91. McKinnon LR, Kaul R. Quality and quantity: mucosal CD4+ T cells and HIV susceptibility. Curr Opin HIV AIDS **2012**; 7:195-202.
92. Hladik F, McElrath MJ. Setting the stage: host invasion by HIV. Nat Rev Immunol **2008**; 8:447-57.
93. Laga M, Manoka A, Kivuvu M, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS **1993**; 7:95-102.
94. Piot P, Laga M. Genital ulcers, other sexually transmitted diseases, and the sexual transmission of HIV. BMJ **1989**; 298:623-4.
95. Masson L, Mlisana K, Little F, et al. Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study. Sex Transm Infect **2014**; 90:580-7.
96. Svanborg C, Godaly G, Hedlund M. Cytokine responses during mucosal infections: role in disease pathogenesis and host defence. Curr Opin Microbiol **1999**; 2:99-105.
97. Arnold KB, Burgener A, Birse K, et al. Increased levels of inflammatory cytokines in the female reproductive tract are associated with altered expression of proteases, mucosal barrier proteins, and an influx of HIV-susceptible target cells. Mucosal Immunol **2016**; 9:194-205.

98. Shey MS, Maharaj N, Archary D, et al. Modulation of Female Genital Tract-Derived Dendritic Cell Migration and Activation in Response to Inflammatory Cytokines and Toll-Like Receptor Agonists. PloS one **2016**; 11:e0155668.
99. McKinnon LR, Nyanga B, Kim CJ, et al. Early HIV-1 infection is associated with reduced frequencies of cervical Th17 cells. J Acquir Immune Defic Syndr **2015**; 68:6-12.
100. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS. Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS **2008**; 22:1493-501.
101. McClelland RS, Lingappa JR, Srinivasan S, et al. Evaluation of the association between the concentrations of key vaginal bacteria and the increased risk of HIV acquisition in African women from five cohorts: a nested case-control study. Lancet Infect Dis **2018**; 18:554-64.
102. McKinnon LR, Achilles SL, Bradshaw CS, et al. The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission. AIDS Res Hum Retroviruses **2019**; 35:219-28.
103. Boris S, Barbes C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect **2000**; 2:543-6.
104. Gosmann C, Anahtar MN, Handley SA, et al. Lactobacillus-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women. Immunity **2017**; 46:29-37.
105. Hayes RJ, Donnell D, Floyd S, et al. Effect of Universal Testing and Treatment on HIV Incidence - HPTN 071 (PopART). N Engl J Med **2019**; 381:207-18.
106. Zambian Ministry of Health. Zambia's master plan towards the elimination of Neglected Tropical Diseases, 2015 - 2020. In: Ministry of Community Development, p. 1-188.
107. Francis SC, Hou Y, Baisley K, et al. Immune Activation in the Female Genital Tract: Expression Profiles of Soluble Proteins in Women at High Risk for HIV Infection. PloS one **2016**; 11:e0143109.
108. Zambian Ministry of Health. Guidelines for the Etiological and Clinical Management of Sexually Transmitted Infections in Zambia, 2017.
109. Corstjens P, de Dood CJ, Knopp S, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Schistosoma Infections-Improvement and Use during SCORE. Am J Trop Med Hyg **2020**; 103:50-7 doi:10.4269/ajtmh.19-0819.
110. Obeng BB, Aryeetey YA, de Dood CJ, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. Annals of tropical medicine and parasitology **2008**; 102:625-33.
111. Pillay P, Taylor M, Zulu SG, et al. Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. Am J Trop Med Hyg **2014**; 90:546-52.
112. Eshleman SH, Piwowar-Manning E, Wilson EA, et al. Determination of HIV status and identification of incident HIV infections in a large, community-randomized trial: HPTN 071 (PopART). J Int AIDS Soc **2020**; 23:e25452.
113. Jespers V, van de Wijgert J, Cools P, et al. The significance of Lactobacillus crispatus and L. vaginalis for vaginal health and the negative effect of recent sex: a cross-sectional descriptive study across groups of African women. BMC Infect Dis **2015**; 15:115.
114. Khalifian S, Raimondi G, Brandacher G. The use of luminex assays to measure cytokines. J Invest Dermatol **2015**; 135:1-5.

115. Luminex Corporation. Luminex MAGPIX. Available at: https://www.luminexcorp.com/eu/research-magpix/ Accessed April 30, 2021.
116. Merck Millipore. Human Cytokine/Chemokine Magnetic Bead Panel Protocol. Available at: https://www.merckmillipore.com/GB/en/product/MILLIPLEX-MAP-Human-Cytokine-Chemokine-Magnetic-Bead-Panel-Immunology-Multiplex-Assay,MM_NF-HCYTOMAG-60K#documentation Accessed April 30, 2021.
117. R&D Systems. Luminex Assay Principles. Available at: "https://www.rndsystems.com/resources/technical/luminex-assay-principle#:~:text=A%20magnet%20in%20the%20MAGPIX,of%20the%20PE%2Dderived%20signal." Accessed April 30, 2021.
118. Masson L, Passmore JA, Liebenberg LJ, et al. Genital inflammation and the risk of HIV acquisition in women. Clin Infect Dis **2015**; 61:260-9.
119. Eckels J, Nathe C, Nelson EK, et al. Quality control, analysis and secure sharing of Luminex(R) immunoassay data using the open source LabKey Server platform. BMC Bioinformatics **2013**; 14:145.
120. Hartung C. Open Data Kit: Tools to Build Information Services for Developing Regions. ICTD 2010 Proceedings of the 4th ACM/IEEE International Conference on Information and Communication Technologies and Development. Available at: https://dl.acm.org/citation.cfm?id=2369236 Accessed April 30, 2021.
121. Jespers V, Crucitti T, van de Wijgert J, et al. A DNA tool for early detection of vaginal dysbiosis in African women. Res Microbiol **2016**; 167:133-41.
122. Boisson S, Engels D, Gordon BA, et al. Water, sanitation and hygiene for accelerating and sustaining progress on neglected tropical diseases: a new Global Strategy 2015-20. Int Health **2016**; 8 Suppl 1:i19-21.
123. Masha SC, Cools P, Sanders EJ, Vaneechoutte M, Crucitti T. Trichomonas vaginalis and HIV infection acquisition: a systematic review and meta-analysis. Sex Transm Infect **2019**; 95:36-42.
124. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A **2011**; 108 Suppl 1:4680-7.
125. Hsieh YJ, Fu CL, Hsieh MH. Helminth-induced interleukin-4 abrogates invariant natural killer T cell activation-associated clearance of bacterial infection. Infect Immun **2014**; 82:2087-97.
126. Han IH, Song HO, Ryu JS. IL-6 produced by prostate epithelial cells stimulated with Trichomonas vaginalis promotes proliferation of prostate cancer cells by inducing M2 polarization of THP-1-derived macrophages. PLoS Negl Trop Dis **2020**; 14:e0008126.
127. Nemati M, Malla N, Yadav M, Khorramdelazad H, Jafarzadeh A. Humoral and T cell-mediated immune response against trichomoniasis. Parasite Immunol **2018**; 40.
128. Cortes-Selva D, Fairfax K. Schistosome and intestinal helminth modulation of macrophage immunometabolism. Immunology **2021**; 162:123-34.
129. Rowley J, Vander Hoorn S, Korenromp E, et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ **2019**; 97:548-62P.
130. McClelland RS, Sangare L, Hassan WM, et al. Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition. The Journal of infectious diseases **2007**; 195:698-702.
131. Fichorova RN. Impact of T. vaginalis infection on innate immune responses and reproductive outcome. J Reprod Immunol **2009**; 83:185-9.

132. Huppert JS, Huang B, Chen C, Dawood HY, Fichorova RN. Clinical evidence for the role of Trichomonas vaginalis in regulation of secretory leukocyte protease inhibitor in the female genital tract. The Journal of infectious diseases **2013**; 207:1462-70.
133. Fichorova RN, Yamamoto HS, Fashemi T, et al. Trichomonas vaginalis Lipophosphoglycan Exploits Binding to Galectin-1 and -3 to Modulate Epithelial Immunity. J Biol Chem **2016**; 291:998-1013.
134. Lin WC, Chang WT, Chang TY, Shin JW. The Pathogenesis of Human Cervical Epithelium Cells Induced by Interacting with Trichomonas vaginalis. PloS one **2015**; 10:e0124087.
135. Hinderfeld AS, Phukan N, Bar AK, Roberton AM, Simoes-Barbosa A. Cooperative Interactions between Trichomonas vaginalis and Associated Bacteria Enhance Paracellular Permeability of the Cervicovaginal Epithelium by Dysregulating Tight Junctions. Infect Immun **2019**; 87.
136. Mercer F, Diala FG, Chen YP, Molgora BM, Ng SH, Johnson PJ. Leukocyte Lysis and Cytokine Induction by the Human Sexually Transmitted Parasite Trichomonas vaginalis. PLoS Negl Trop Dis **2016**; 10:e0004913.
137. Fichorova RN, Buck OR, Yamamoto HS, et al. The villain team-up or how Trichomonas vaginalis and bacterial vaginosis alter innate immunity in concert. Sex Transm Infect **2013**; 89:460-6.
138. Dailey DC, Chang TH, Alderete JF. Characterization of Trichomonas vaginalis haemolysis. Parasitology **1990**; 101 Pt 2:171-5.
139. Thurman AR, Doncel GF. Innate immunity and inflammatory response to Trichomonas vaginalis and bacterial vaginosis: relationship to HIV acquisition. Am J Reprod Immunol **2011**; 65:89-98.
140. Gosselin A, Monteiro P, Chomont N, et al. Peripheral blood CCR4+CCR6+ and CXCR3+CCR6+CD4+ T cells are highly permissive to HIV-1 infection. J Immunol **2010**; 184:1604-16.
141. Lederman MM, Penn-Nicholson A, Cho M, Mosier D. Biology of CCR5 and its role in HIV infection and treatment. JAMA **2006**; 296:815-26.
142. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. Nat Rev Immunol **2011**; 11:375-88.
143. Mutapi F, Winborn G, Midzi N, Taylor M, Mduluza T, Maizels RM. Cytokine responses to Schistosoma haematobium in a Zimbabwean population: contrasting profiles for IFN-gamma, IL-4, IL-5 and IL-10 with age. BMC Infect Dis **2007**; 7:139.
144. Lyke KE, Dabo A, Sangare L, et al. Effects of concomitant Schistosoma haematobium infection on the serum cytokine levels elicited by acute Plasmodium falciparum malaria infection in Malian children. Infect Immun **2006**; 74:5718-24.
145. Fu CL, Odegaard JI, Herbert DR, Hsieh MH. A novel mouse model of Schistosoma haematobium egg-induced immunopathology. PLoS Pathog **2012**; 8:e1002605.
146. King CL, Malhotra I, Mungai P, et al. Schistosoma haematobium-induced urinary tract morbidity correlates with increased tumor necrosis factor-alpha and diminished interleukin-10 production. The Journal of infectious diseases **2001**; 184:1176-82.
147. Odegaard JI, Hsieh MH. Immune responses to Schistosoma haematobium infection. Parasite Immunol **2014**; 36:428-38.
148. Pearce EJ, Caspar P, Grzych JM, Lewis FA, Sher A. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. J Exp Med **1991**; 173:159-66.

149. Mishra PK, Palma M, Bleich D, Loke P, Gause WC. Systemic impact of intestinal helminth infections. Mucosal Immunol **2014**; 7:753-62.
150. Erikstrup C, Kallestrup P, Zinyama-Gutsire RB, et al. Schistosomiasis and infection with human immunodeficiency virus 1 in rural Zimbabwe: systemic inflammation during co-infection and after treatment for schistosomiasis. Am J Trop Med Hyg **2008**; 79:331-7.
151. Leutscher PD, Pedersen M, Raharisolo C, et al. Increased prevalence of leukocytes and elevated cytokine levels in semen from Schistosoma haematobium-infected individuals. The Journal of infectious diseases **2005**; 191:1639-47.
152. Bustinduy AL, Sutherland LJ, Chang-Cojulun A, et al. Age-Stratified Profiles of Serum IL-6, IL-10, and TNF-alpha Cytokines Among Kenyan Children with Schistosoma haematobium, Plasmodium falciparum, and Other Chronic Parasitic Co-Infections. Am J Trop Med Hyg **2015**; 92:945-51.
153. Abbas AK Lichtman AH Pillai S. (2018). Cellular and molecular immunology (9th ed.). Philadelphia: Saunders Elsevier.
154. Del Prete G. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. Allergy **1992**; 47:450-5.
155. Fedak KM, Bernal A, Capshaw ZA, Gross S. Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol **2015**; 12:14.
156. Barlow JT, Bogatyrev SR, Ismagilov RF. A quantitative sequencing framework for absolute abundance measurements of mucosal and lumenal microbial communities. Nat Commun **2020**; 11:2590.
157. Norseth HM, Ndhlovu PD, Kleppa E, et al. The colposcopic atlas of schistosomiasis in the lower female genital tract based on studies in Malawi, Zimbabwe, Madagascar and South Africa. PLoS Negl Trop Dis **2014**; 8:e3229.
158. Amoah AS, Hoekstra PT, Casacuberta-Partal M, et al. Sensitive diagnostic tools and targeted drug administration strategies are needed to eliminate schistosomiasis. The Lancet Infectious Diseases **2020**; 20:e165-e72.
159. Grimes JE, Croll D, Harrison WE, Utzinger J, Freeman MC, Templeton MR. The relationship between water, sanitation and schistosomiasis: a systematic review and meta-analysis. PLoS Negl Trop Dis **2014**; 8:e3296.
160. Archary D, Liebenberg LJ, Werner L, et al. Randomized Cross-Sectional Study to Compare HIV-1 Specific Antibody and Cytokine Concentrations in Female Genital Secretions Obtained by Menstrual Cup and Cervicovaginal Lavage. PloS one **2015**; 10:e0131906.
161. Polman NJ, Ebisch RMF, Heideman DAM, et al. Performance of human papillomavirus testing on self-collected versus clinician-collected samples for the detection of cervical intraepithelial neoplasia of grade 2 or worse: a randomised, paired screen-positive, non-inferiority trial. Lancet Oncol **2019**; 20:229-38.
162. Francis SC, Holm Hansen C, Irani J, et al. Results from a cross-sectional sexual and reproductive health study among school girls in Tanzania: high prevalence of bacterial vaginosis. Sex Transm Infect **2019**; 95:219-27.
163. Hegertun IE, Sulheim Gundersen KM, Kleppa E, et al. S. haematobium as a common cause of genital morbidity in girls: a cross-sectional study of children in South Africa. PLoS Negl Trop Dis **2013**; 7:e2104.
164. Downs JA, Kabangila R, Verweij JJ, et al. Detectable urogenital schistosome DNA and cervical abnormalities 6 months after single-dose praziquantel in women with Schistosoma haematobium infection. Trop Med Int Health **2013**; 18:1090-6.

165. Srinivasan S, Liu C, Mitchell CM, et al. Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. PloS one **2010**; 5:e10197.
166. Gallo MF, Warner L, King CC, et al. Association between semen exposure and incident bacterial vaginosis. Infect Dis Obstet Gynecol **2011**; 2011:842652.
167. Hayes R, Ayles H, Beyers N, et al. HPTN 071 (PopART): rationale and design of a cluster-randomised trial of the population impact of an HIV combination prevention intervention including universal testing and treatment - a study protocol for a cluster randomised trial. Trials **2014**; 15:57.
168. Kalinda C, Chimbari MJ, Mukaratirwa S. Schistosomiasis in Zambia: a systematic review of past and present experiences. Infect Dis Poverty **2018**; 7:41.
169. Knopp S, Person B, Ame SM, et al. Elimination of schistosomiasis transmission in Zanzibar: baseline findings before the onset of a randomized intervention trial. PLoS Negl Trop Dis **2013**; 7:e2474.
170. Agnew-Blais J, Carnevale J, Gropper A, Shilika E, Bail R, Ngoma M. Schistosomiasis haematobium prevalence and risk factors in a school-age population of peri-urban Lusaka, Zambia. J Trop Pediatr **2010**; 56:247-53.
171. Rutty Phiri C, Sturt AS, Webb EL, et al. Acceptability and feasibility of genital self-sampling for the diagnosis of female genital schistosomiasis: a cross-sectional study in Zambia. Wellcome Open Res **2020**; 5:61.
172. International Agency for Research on Cancer. Schistosomes, Liver Flukes, and Helicobacter pylori. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. 1994; vol 61. Available at: https://www.ncbi.nlm.nih.gov/books/NBK487782/pdf/Bookshelf_NBK487782.pdf. Accessed April 30, 2021.
173. Yi TJ, Shannon B, Prodger J, McKinnon L, Kaul R. Genital immunology and HIV susceptibility in young women. Am J Reprod Immunol **2013**; 69 Suppl 1:74-9.
174. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis **2020**; 71:1367-76.
175. Ezeamama AE, Bustinduy AL, Nkwata AK, et al. Cognitive deficits and educational loss in children with schistosome infection-A systematic review and meta-analysis. PLoS Negl Trop Dis **2018**; 12:e0005524.
176. Bustinduy AL, Wright S, Joekes EC, et al. One hundred years of neglect in paediatric schistosomiasis. Parasitology **2017**; 144:1613-23.
177. World Health Organization. Schistosomiasis and soil-transmitted helminthiasis: number of people treated in 2016. Available at: https://www.who.int/neglected_diseases/resources/who_wer9249/en/. Accessed April 30, 2021.
178. World Health Organization. Ending the neglect to attain the Sustainable Development Goals A road map for Neglected Tropical Diseases 2021-2030. Available at: https://www.who.int/publications/i/item/9789240010352. Accessed April 30, 2021.
179. Friedman JF, Olveda RM, Mirochnick MH, Bustinduy AL, Elliott AM. Praziquantel for the treatment of schistosomiasis during human pregnancy. Bull World Health Organ **2018**; 96:59-65.
180. UNAIDS. No more neglect — Female genital schistosomiasis and HIV — Integrating sexual and reproductive health interventions to improve women's lives, 2019. Available at:

https://www.unaids.org/en/resources/documents/2019/female_genital_schistosomiasis_and_hiv Accessed April 30, 2021.
181. UNAIDS. Prevailing against pandemics by putting people at the centre - World AIDS Day report 2020. Available at: https://www.unaids.org/en/resources/documents/2020/prevailing-against-pandemics Accessed April 30, 2021.
182. Land KJ, Boeras DI, Chen XS, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol **2019**; 4:46-54.
183. Archer J, Barksby R, Pennance T, et al. Analytical and Clinical Assessment of a Portable, Isothermal Recombinase Polymerase Amplification (RPA) Assay for the Molecular Diagnosis of Urogenital Schistosomiasis. Molecules **2020**; 25.
184. UNAIDS. End Inequalities. End AIDS. Global AIDS strategy 2021-2026. Available at: https://www.unaids.org/sites/default/files/PCBSS_Global_AIDS_Strategy_2021--2026_EN.pdf Accessed April 30, 2021.
185. Kjetland EF, Leutscher PD, Ndhlovu PD. A review of female genital schistosomiasis. Trends in parasitology **2012**; 28:58-65.
186. Selhorst P, Masson L, Ismail SD, et al. Cervicovaginal Inflammation Facilitates Acquisition of Less Infectious HIV Variants. Clin Infect Dis **2017**; 64:79-82.

Appendices

Appendix 1 – The BILHIV Study

Citation: Sturt AS, Webb EL, Phiri CR, et al. Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. PLoS Negl Trop Dis **2020**; 14:e0008337.

RESEARCH ARTICLE

Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study

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Citation: Sturt AS, Webb EL, Phiri CR, Mweene T, Chola N, van Dam GJ, et al. (2020) Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study. PLoS Negl Trop Dis 14(7): e0008337. https://doi.org/10.1371/journal.pntd.0008337

Editor: Piet Cools, Ghent University, BELGIUM

Received: December 5, 2019

Accepted: April 29, 2020

Published: July 14, 2020

Data Availability Statement: Due to the sensitive nature of the data collected in the BILHIV study, data will be available upon request. The data will be available on LSHTM Data Compass. Data will available on request, which is advised by the LSHTM information management team. The data will be available by request on LSHTM Data Compass.

Funding: ALB is supported by a grant from the Wellcome Trust (205954/Z/17/Z) https://wellcome.ac.uk. ELW and RH received salary funding from

Abstract

Background

Given the potentially causal association of female genital schistosomiasis (FGS) with HIV-1 infection, improved diagnostics are urgently needed to scale-up FGS surveillance. The BILHIV (bilharzia and HIV) study assessed the performance of home-based self-collection methods (cervical and vaginal swabs) compared to cervicovaginal lavage (CVL) for the detection of *Schistosoma* DNA by real-time polymerase chain reaction (PCR).

Methods

Between January and August 2018, a consecutive series of female participants from the Population-Cohort of the previous HIV prevention trial HPTN 071 (PopART), resident in Livingstone, Zambia were invited to take part in BILHIV if they were 18–31 years old, non-pregnant and sexually active. Genital self-collected swabs and a urine specimen were obtained and a questionnaire completed at home visits. CVL was obtained at clinic follow-up.

Results

603 women self-collected genital swabs. Of these, 527 women had CVL performed by a mid-wife during clinic follow-up. *Schistosoma* DNA was more frequently detected in genital self-collected specimens (24/603, 4.0%) compared to CVL (14/527, 2.7%). Overall, 5.0% (30/603) women had female genital schistosomiasis, defined as a positive PCR by any genital sampling method (cervical swab PCR, vaginal swab PCR, or CVL PCR) and 95% (573/

MRC Grant Reference MR/K012126/1. This award is jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and is also part of the EDCTP2 program supported by the European Union. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

603) did not have a positive genital PCR. The sensitivity of any positive genital self-collected swab against CVL was 57.1% (95% CI 28.9–82.3%), specificity 97.3% (95.5–98.5%). In a subset of participants with active schistosome infection, determined by detectable urine Circulating Anodic Antigen (CAA) (15.1%, 91/601), positive PCR (4.3%, 26/601), or positive microscopy (5.5%, 33/603), the sensitivity of any positive self-collected specimen against CVL was 88.9% (51.8–99.7%).

Conclusions

Genital self-sampling increased the overall number of PCR-based FGS diagnoses in a field setting, compared with CVL. Home-based sampling may represent a scalable alternative method for FGS community-based diagnosis in endemic resource limited settings.

Author summary

Female Genital schistosomiasis (FGS) is a neglected and disabling disease that results when eggs from the waterborne parasite *Schistosoma haematobium* are trapped in the human reproductive tract. Current female genital schistosomiasis (FGS) diagnostic strategies are limited because they require expertise and equipment that may not be readily available in low income settings. Improved and accessible diagnostics are urgently needed to scale-up FGS surveillance. This is especially important as FGS has been associated with HIV-1 infection. The BILHIV (bilharzia and HIV) study assessed the performance of home-based self-collection methods (cervical and vaginal swabs) compared with a clinic-based cervicovaginal lavage (CVL) performed by a medical professional. Both methods used real-time polymerase chain reaction (PCR) to detect *Schistosoma* DNA. We found that, in a field setting, self-collected genital and cervical swabs increased the overall number of PCR-based FGS diagnoses, compared with clinically collected CVL. We report the sensitivity of self-collected swabs for the diagnosis of FGS, compared with CVL. We found that the sensitivity of self-collected genital swabs was high in a subset of women with active schistosome infection. We suggest that home-based self-sampling may represent a scalable community-based sampling platform for FGS community-based diagnosis in endemic resource limited settings.

Introduction

An estimated 82 million women in sub-Saharan Africa live with *Schistosoma (S.) haematobium* or *S. mansoni* infections [1] that follow fresh water contact. In Zambia alone, it is estimated that 3.8 million people (approximately 22% of the population) are infected with *Schistosoma* species [2]. After maturation, these trematode parasites commence egg-laying in the host's venous system and disease occurs when tissue-entrapped eggs cause both local and systemic pathology [3]. Female genital schistosomiasis (FGS), defined as the detection of eggs or *Schistosoma spp*. DNA in genital tissue or fluids [4], affects an estimated 20–56 million women worldwide [5], mostly in sub-Saharan Africa, including Zambia [6]. The presence of eggs or *Schistosoma spp*. DNA in urine and stool does not confirm concurrent genital involvement [7, 8].

Egg deposition in FGS occurs in clusters [9], often at the squamocolumnar junction in the subepithelial connective tissue [9] and is therefore frequently missed on superficial Papanicolaou-smear based sampling [8–10]. Often tissue-lodged eggs are accompanied by characteristic sandy patches (both grainy and homogenous) [11] and rubbery papules [12]. However, in up to one-quarter of cases, FGS occurs in macroscopically normal appearing cervical tissue [9, 13]. Classically, FGS lesions are visualized colposcopically [8, 12], requiring equipment and trained clinical expertise that may not be available in low income settings [4]. Also, concern that tissue biopsy, the proposed reference standard, may provide a means of entry for HIV-1 infection [4] has limited its use in FGS research. Given this theoretical risk, the use of polymerase chain reaction (PCR) on cervicovaginal lavage (CVL) has been advocated as an acceptable and less-invasive method of FGS diagnosis in research settings [8, 12, 14, 15]. However, because CVL sampling requires a trained health professional, vaginal speculum insertion, and an intact cold chain, it is not likely scalable for population–based FGS surveillance.

FGS has been associated with adverse reproductive health outcomes, such as infertility [16], ectopic pregnancy [17, 18], and abortion [17]. Eggs deposited in reproductive tissues release immunogenic antigens [19], resulting in granulomatous reaction and fibrosis [18]. There is also increasing evidence on the association of FGS with prevalent HIV-1 infection [10, 20], a relationship that may be causal. Accurate, accessible, and affordable diagnostics are urgently needed to scale-up FGS surveillance and treatment. Schistosome infection is associated with a continuum of disease co-morbidities and post-infection complications [21]. FGS can occur along this continuum and there is currently not a diagnostic test that captures the entire range of FGS clinical presentations [21].

Self-sampling has been used for diagnosis of reproductive tract infections, including human papillomavirus (HPV) [22]. Compared with clinician collected specimens, self-collected PCR-based HPV testing is acceptable to participants [23], increases screening coverage [22, 24], and is as sensitive as clinician collected samples [25]. A home-based diagnostic option for FGS with decreased reliance on medical professionals would have high field applicability. This study was designed to compare the performance of two home-based self-collection methods (cervical and vaginal swabs), to clinic-based, midwife-collected CVL for the detection of *Schistosoma* DNA by Nucleic Acid Amplification Tests (NAATs).

Methods

Study site and subjects

Between January and August 2018, participants from the Population Cohort (PC) of HPTN 071 (PopART), a trial to measure the impact of an HIV combination prevention package, including universal HIV test and treat [26], were recruited to participate in the cross-sectional bilharzia and HIV (BILHIV) study. Women were eligible to participate if they were 18–31 years old, non-pregnant, sexually active, and resident in one of the two communities (designated Community-A and Community-B) that participated in HPTN 071 (PopART) in Livingstone, Zambia. Central Livingstone is located within 5–10 kilometres of the Zambezi River, with a tributary flowing in close proximity to Community A. HPTN 071 (PopART) is a cluster randomized trial including 21 participating communities. At the community level, the Population Cohort included one randomly selected adult 18 to 44 years of age from a random sample of households in each community [26]. BILHIV study participants were then selected as a consecutive sample from the HPTN 071 (PopART) Population Cohort. A 2013 survey of school aged children in Livingstone reported prevalence ranges for urinary *S. haematobium* infection between 3.3% and 73.3% (median 15.0%, mean 23.3%) [27].

Home-based sample collection and questionnaire

Trained community workers provided home visits to women who gave an "expression of interest" in the BILHIV study at the HPTN 071 (PopART) PC 36-month visit. The home visit included assessment of eligibility, a questionnaire, genital self-sampling (cervical and vaginal), and a single urine specimen. Trained field workers provided study information in the participant's preferred language. Following written informed consent, a questionnaire containing questions regarding demographics, water contact, sexual behaviour, and genital symptoms was administered. There were no restrictions on the timing of urine sample self-collection, and 69.5% (419/603) were performed between 9:00 and 14:00. The community worker provided participating women with instructions for urine collection and cervical and vaginal self-sampling. Participants were instructed to hold a 6-inch PrimeSwab (Longhorn Diagnostics, Texas, USA) at the 2 3/8-inch score mark, insert the swab vaginally until their fingers touched the labia minora, and rotate the swab against the vaginal walls (minimum 15 repetitions) (Fig 1). Similarly, for the cervical swab, participants were instructed to hold a 6 3/4-inch flocked swab (Miraclean, Shenzen, China) with a quadrilateral kite-shaped tip at the non-flocked end, insert the swab until their fingers touched the labia minora and/or encountered resistance, and rotate the swab against the place of resistance (minimum 15 repetitions). Each flocked swab head was placed in individual screw cap microtubes (STARLAB, Hamburg, Germany) by the participant after the swab shaft was broken. Both swab specimens and urine were placed in cool boxes for transportation to the laboratory for further processing (see Supplementary Material). Women with evidence of active schistosome infection, defined by any positive urine examination (microscopy, Circulating Anodic Antigen (CAA) or PCR), or women with clinical evidence of FGS as determined by the midwife's clinical examination [28], were treated free of charge with 40 mg/kg praziquantel, either at the clinic visit, or via community workers.

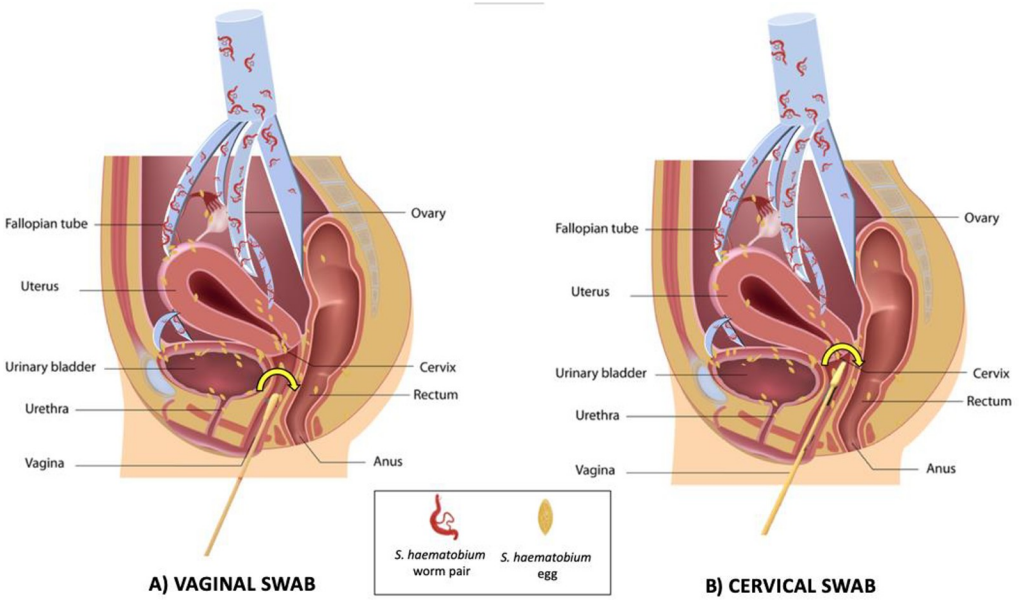

Fig 1. Genital Self-sampling in the BILHIV Study. A) Participants held a 6-inch vaginal swab at the 2 3/8-inch score mark, inserted the swab vaginally until their fingers touched the labia minora, and rotated the swab against the vaginal walls (minimum 15 repetitions). B) Participants held the 6 3/4-inch cervical swab at the non-flocked end, inserted the swab until their fingers touched the labia minora and/or encountered resistance, and rotated the swab against the place of resistance (minimum 15 repetitions).

https://doi.org/10.1371/journal.pntd.0008337.g001

Clinic-based sample collection

Enrolled women who were not currently menstruating were invited to attend Livingstone Central Hospital cervical cancer screening clinic within days of self-sampling, where one of two trained midwives performed a cervicovaginal lavage (CVL). After speculum insertion, normal saline (10mL) was flushed continuously with a bulb syringe across the cervix and vaginal walls for one minute and collected from the posterior fornices. Images of the vagina and cervix were captured with a point-of-care colposcope (MobileODT, Tel Aviv, Israel). Participants with suspected reproductive tract infections were offered syndromic management, as per the Ministry of Health [29]. Routine testing for reproductive tract infections was not performed in this study.

Laboratory analysis

Urine aliquoting for quantification of CAA, PCR, and urinalysis were performed on the day of specimen arrival at the laboratory (see S1 Text). The remaining urine, up to 60mL per participant, was centrifuged in 15mL aliquots and examined by microscopy within 24 hours. The pellet from each 15mL urine aliquot (5 maximum) was evaluated for *S. haematobium* eggs. When a pellet contained at least one terminal-spined ovum, the participant was considered positive and the total number of counted eggs in the pellet was reported. Review of all positive and 10% of the negative specimens was conducted blinded by an expert for quality control. Dipsticks were used for analysis of hematuria and proteinuria (Multistix, Siemens, Germany). An up-converting reporter particle (UCP) lateral flow (LF) assay for the quantification of CAA in urine (0.4 mL) was performed at the Leiden University Medical Center (LUMC) (see S1 Text) [30]. Urine CAA antigen levels are known to reflect adult worm burden and decline after successful treatment with praziquantel [31, 32].

PCR for *Schistosoma spp*

Pre-treatment and DNA isolation of CVL, genital swabs, and urine samples were performed (see S1 Text) in different sites at the LUMC, hence minor differences in the laboratory procedures. Briefly, genital swabs were suspended in PBS and vortexed, and thereafter handled as lavages, by pre-treating using a proteinase K heating step and isolating DNA using QIAamp spin columns (QIAGEN Benelux, Venlo, The Netherlands) [8, 12, 33]. Two hundred μL of urine sample per participant were pretreated using Precellys Soil grinding SK38 (Bertin technology, Montigny-le-Bretonneux, France) and DNA was isolated using MagNA Pure 96 technology (Roche Diagnostics, Penzberg, Germany). Internal transcribed spacer 2-based real-time PCR was performed as previously described [33], with slight modifications (see S1 Text). DNA amplification and detection were performed with the CFX96 Real Time PCR Detection System (BioRad, California, USA). The output in threshold cycles (C_t) was analysed using BioRad CFX software. Negative and positive control samples were included in each amplification run. Parasite DNA load is categorized by the following pre-specified C_t threshold for all specimens processed by PCR (urine, cervical swab, vaginal swab, and CVL): any C_t-value observed means positive and no C_t-value observed means negative (S1 Text) [34]. All specimens tested by PCR at LUMC were blinded for clinical and microscopy data.

Ethical considerations

The study was approved by the University of Zambia Biomedical Research Ethics Committee (reference 011-08-17), the Zambia National Health Research Authority and the London School of Hygiene and Tropical Medicine Ethics Committee (reference 14506). Permission to

conduct the study was given by Livingstone District health office and the superintendent of Livingstone Central Hospital. All the human subjects in this study were adults.

Statistical methods

We anticipated that the prevalence of *S. haematobium* infection would be 30% and that PCR performed on self-collected specimens would have sensitivity of 70% and specificity of 85%. Under these assumptions a sample size of 600 women would allow us to detect these target values with 95% confidence intervals of 63%-77% and 81%-88%, respectively.

Data were entered on hand-held electronic data capture devices using Open Data Kit and analysed using STATA 15.1 (Stata Corporation, College Station, TX). Continuous variables were summarized by median and interquartile range (IQR), and categorical variables by frequency and percentage. Differences in participant characteristics between the two communities were evaluated using Fisher's exact, chi-squared, and Wilcoxon-Mann-Whitney tests. The association between age group and positive test result was assessed using the test for trend. For the comparison of home-based self-collection methods with clinic based CVL, we calculated sensitivity and specificity. There weren't indeterminate PCR results or missing data for genital self-collected swabs (the index test). "Definite" FGS was defined as any positive genital PCR (cervical swab, vaginal swab or CVL). The presence of either CAA or *S. haematobium* eggs in clinical specimens indicates the presence of infection with viable worms [30]. Thus, in this study, "active" schistosome infection refers to participants with a positive urine PCR, CAA, or microscopy. To evaluate if sequential use of FGS diagnostics might be beneficial, an ad-hoc secondary analysis was performed in which calculation of the diagnostic performance was restricted to those with an active schistosome infection according to positive urine PCR, CAA, or microscopy. CVL was chosen as the *a priori* reference standard for the BILHIV study. In the absence of a universally recognized reference standard for FGS, a composite FGS outcome was constructed, defined as a positive result on any genital PCR specimen (swab or CVL).

Results

Overall, 1105 women in the study communities from the HPTN 071 (PopART) Population Cohort met the inclusion criteria. A total of 603 (54.5%) eligible women were enrolled with 527 (87.0%) completing clinic follow up (Fig 2). Women from both communities reported regular water contact in childhood (p = 0.22) (Table 1). Overall, an active schistosome infection was diagnosed in 15.6% (94/601) (Table 2), of which 33 were urine microscopy positive and an additional 61 cases were diagnosed by urine CAA. Urine PCR confirmed the presence of schistosome infection in 26 participants and did not detect any additional cases. Overall, 15.6% (94/601) had schistosome infection (Table 2). The prevalence of *S. haematobium* infection was 5.5% (33/603) based on urine microscopy, 15.1% (91/601) by urine CAA, and 4.3% (26/601) by urine PCR (Table 2).

There were 30 women (5.0%) with definite FGS, defined as any positive genital PCR (cervical swab, vaginal swab or CVL), 22/291 (7.6%) in Community-A and 8/238 (3.4%) in Community-B (p = 0.04) and 95% (573/603) did not have definite FGS. The proportion of participants testing positive decreased with increasing age for all tests except urine CAA and CVL PCR (urine microscopy p-trend = 0.004, urine PCR p-trend = 0.003, any PCR-positive genital specimen p-trend<0.001, cervical swab PCR p-trend<0.001, vaginal swab PCR p-trend<0.001) (Fig 3 & S1 Table). In the 30 women with FGS, the prevalence of active schistosome infection was 70.0% (21/30). Of the 94 women with an active schistosome infection, 22.3% (21/94) had FGS and of the 507 women without active schistosome infection 1.8% (9/507) had FGS (p<0.001), these numbers include women who did not attend clinic follow-up. Fig 4 illustrates

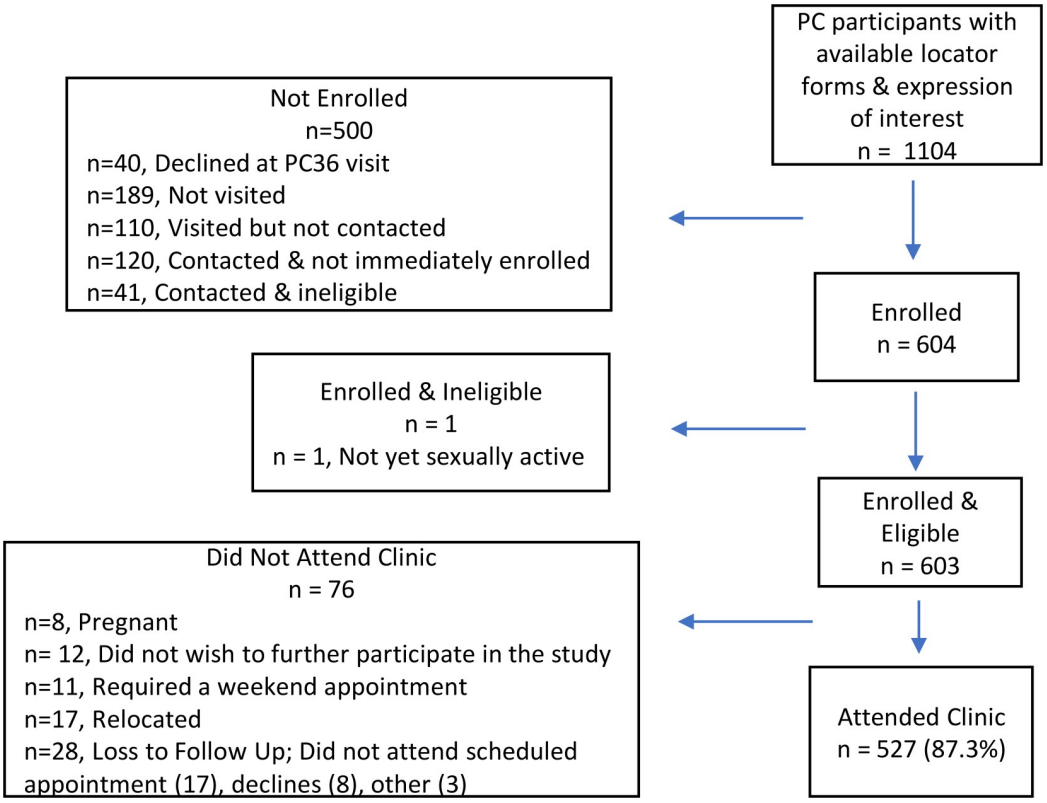

Fig 2. BILHIV study flow diagram. Not visited - the participant was not visited before the study closed for enrollment (189) Visited but not contacted - a visit was made to the study household, but the participant could not be located (70), had relocated (39), or died (1) Contacted & not immediately enrolled - (42), out of town (18), declined to participate (60) Contacted & ineligible - virgin (16), pregnant (17), over 31 (8)

https://doi.org/10.1371/journal.pntd.0008337.g002

FGS diagnosis based on PCR positivity for each of the three genital sampling methods. No adverse events were reported in either group related to use of the index or reference test.

The sensitivity of a positive result from any positive self-collected specimen (cervical or vaginal swab) against CVL was 57.1% (28.9–82.3) with specificity of 97.3% (95.5–98.5%) (Table 3). Compared with CVL, the sensitivity of self-collected cervical swabs (42.9% [17.7–71.1%]) was somewhat higher than for vaginal swabs (35.7% [12.8–64.9]), albeit with wide confidence intervals. The specificity of PCR in self-collected specimens versus CVL was high regardless of sampling technique (cervical 97.7% [95.9–98.8%], vaginal 98.2% [96.7–99.2%]) (Table 3). Self-collected genital swabs were also evaluated as the reference standard (Table 3). Of these comparisons, the highest sensitivity (80% [61.4–92.3] was achieved using combined swab results compared with the composite FGS diagnosis (Table 3).

In a secondary analysis of those participants diagnosed with an active schistosome infection, the sensitivity of PCR from any positive self-collected swab specimen (cervical or vaginal) against CVL was marginally higher than in the primary analysis, albeit with wide confidence intervals (Table 4). The gain in sensitivity when evaluating those participants diagnosed with an active schistosome infection was accompanied by a decline in specificity (Table 4).

Discussion

The BILHIV study is the first to examine the performance of self-collected genital swabs (vaginal and cervical) for the diagnosis of FGS. Self-collected swabs were compared with provider-

Table 1. Baseline characteristics of 603 Zambian women living in *S. haematobium* endemic areas near the Zambezi river by district.

Characteristics		Overall (n = 603)	Community A (n = 319)	Community B (n = 284)	p-value*
Age in years–Median (IQR)		24 (22–28)	26 (23–29)	24 (21–27)	<0.001
Marital Status	Single	258 (42.8%)	110 (34.5%)	148 (52.1%)	<0.001
	Married or Cohabitating	320 (53.1%)	193 (60.5%)	127 (44.7%)	
	Divorced or Separated	23 (3.8%)	15 (4.7%)	8 (2.8%)	
	Widowed	2 (0.3%)	1 (0.3%)	1 (0.4%)	
Education (highest level)	Any Primary School	167 (27.7%)	117 (36.7%)	50 (17.6%)	<0.001
	Any Secondary School	364 (60.4%)	173 (54.2%)	191 (67.3%)	
	Training in a Trade	59 (9.8%)	20 (6.3%)	39 (13.7%)	
	Degree or Higher	3 (0.5%)	3 (0.9%)	0 (0.0%)	
	None	10 (1.7%)	6 (1.9%)	4 (1.4%)	
Employment Status	Working	408 (67.7%)	200 (62.7%)	208 (73.2%)	0.006
	Not Working	195 (32.3%)	119 (37.3%)	76 (26.8%)	
Current Water Contact	None	512 (84.9%)	263 (82.5%)	249 (87.7%)	0.02
	At Least Weekly	18 (3.0%)	11 (3.5%)	7 (2.5%)	
	Every 1–2 Months	30 (5.0%)	24 (7.5%)	6 (2.1%)	
	Every 6–12 Months	43 (7.1%)	21 (6.6%)	22 (7.8%)	
Childhood Water Contact	None	186 (30.9%)	96 (30.1%)	90 (31.7%)	0.22
	At Least Weekly	381 (63.2%)	208 (65.2%)	173 (60.9%)	
	Every 1–2 Months	24 (4.0%)	12 (3.8%)	12 (4.2%)	
	Every 6–12 Months	12 (2.0%)	3 (0.9%)	9 (3.2%)	
Self-reported History of Schistosomiasis	No	572 (94.8%)	294 (92.2%)	278 (97.9%)	0.006
	Yes	25 (4.2%)	20 (6.3%)	5 (1.8%)	
	Maybe	6 (1.0%)	5 (1.6%)	1 (0.4%)	
Self- reported Treatment with Praziquantel	No	523 (86.7%)	270 (84.6%)	253 (89.1%)	0.11
	Yes	61 (10.1%)	40 (12.5%)	21 (7.4%)	
	Maybe	19 (3.2%)	9 (2.8%)	10 (3.5%)	

* comparison of Community-A vs Community-B

https://doi.org/10.1371/journal.pntd.0008337.t001

collected CVL, an accepted, non-invasive standard for FGS diagnosis in research settings [12, 14]. The addition of self-collected swabs to CVL increased the number of FGS diagnoses. The sensitivity of any self-collected genital specimen compared with CVL improved when only those diagnosed with an active infection (i.e. positive urine CAA, urine PCR, or urine microscopy) were considered. Using a composite definition of FGS (any PCR positive genital specimen) as the reference standard also improved the sensitivity of self-collected genital specimens. In the absence of a reference standard for FGS [21], the specificity of genital swabs is limited by comparison with CVL alone. CVL for FGS diagnosis has imperfect sensitivity and may not identify all true positives. In this analysis, the sensitivity of CVL as the index test compared to self-sampling as the reference was similar to that of self-sampling as the index test with CVL as reference standard.

A sub-analysis of the BILHIV data also suggests that sensitivity of PCR-based testing of genital self-samples is high in the subgroup of participants with active infection. The cost of genital swabs (0.50$/vaginal swab and 0.30$/cervical swab) and molecular testing (6.68$/test) may be

Table 2. Diagnostic test results and FGS prevalence in 603 Zambian women living in *S. haematobium* endemic areas near the Zambezi river.

Characteristics	Overall (n = 603) % (n)	Community-A (n = 319) % (n)	Community-B (n = 284) % (n)	p-value
Hematuria	28.7 (173)	31.0 (99)	26.1 (74)	0.2
Urine Microscopy	5.5 (33/603)	8.2 (26/319)	2.5 (7/284)	0.002
Median egg count per 15mL‡	18	17.5	18	
IQR	5–35	5–35	8–90	
Urine CAA*	15.1 (91/601)	20.5 (65/317)	9.2 (26/284)	<0.001
Median pg/mL‡	9.10	11.46	5.93	
IQR	2.2–58.6	2.3–42.1	2.2–112.0	
Urine PCR*	4.3 (26/601)	6.3 (20/317)	2.1 (6/284)	0.012
Median Ct‡	29.6	30.2	29.3	
IQR	27.8–33.5	26.4–34.9	29.0–32.7	
Active schistosome Infection* *[any urine test positive]*	15.6 (94/601)	21.1 (67/317)	9.5 (27/284)	<0.001
PCR–CVL†	2.7 (14/527)	3.8 (11/290)	1.3 (3/237)	0.06**
Median Ct‡	34.6	35.3	33.2	
IQR	33.0–37.0	33.0–37.0	21.0–38.0	
PCR–Cervical Swab	3.3 (20/603)	4.4 (14/319)	2.1 (6/284)	0.12
Median Ct‡	35.3	35.8	33.3	
IQR	29.6–37.1	29.4–37.2	29.7–36.3	
PCR–Vaginal Swab	2.5 (15/603)	2.5 (8/319)	2.5 (7/284)	0.97
Median Ct‡	34.3	35.4	32.2	
IQR	23.6–37.1	30.6–37.2	23.2–35.2	
Any PCR Positive Sample††	5.7 (30/529)	7.6 (22/291)	3.4 (8/238)	0.02
PCR positive–Vaginal or Cervical Swab	4.0 (24/603)	5.3 (17/319)	2.5 (7/284)	0.07

*2 urine vials arrived at LUMC empty (n = 601)

** Calculated by Fisher's exact (otherwise by chi-squared)

† 527 women presented for CVL– 290 Community-A and 237 Community-B

‡ Median egg count, CAA concentrations and PCR Ct-value restricted to positive tests

††One participant from each of Site-1 and Site-2 had positive self-collected PCR specimens but did not present to clinic (n = 529)

Abbreviations: CAA—Circulating Anodic Antigen, CVL–Cervicovaginal Lavage, PCR–Polymerase Chain Reaction for the detection of *Schistosoma* DNA

https://doi.org/10.1371/journal.pntd.0008337.t002

affordable in some research settings, but more field-appropriate and scalable NAAT methods should be investigated. Given the cost of FGS diagnostics, an algorithm which advocates FGS screening in resource-limited settings among women with positive CAA or urine microscopy could assist in conserving resources, labour, reagents, and other costs. Applying this algorithm to the BILHIV study identifies 70.0% of FGS cases identified in a relatively low-prevalence area. However, an important limitation of restricting genital self-sampling to a sub-group with active infection is a decreased ability to detect FGS cases in the population, as FGS would not be identified in women without active infection. This decrement in the overall sensitivity of screening must be balanced against the availability of resources. Other practical limitations to this strategy include low prevalence populations, the imperfect sensitivity of *Schistosoma* PCR in genital self-sampling, and the current availability of PCR and CAA as research tools that do not yet allow individual rapid diagnosis at the point-of-care [35]. To maximally leverage resources, FGS self-sampling would be integrated into other reproductive health platforms, ideally those that also utilize self-sampling in the diagnosis of reproductive tract infections, such as HPV [22, 25].

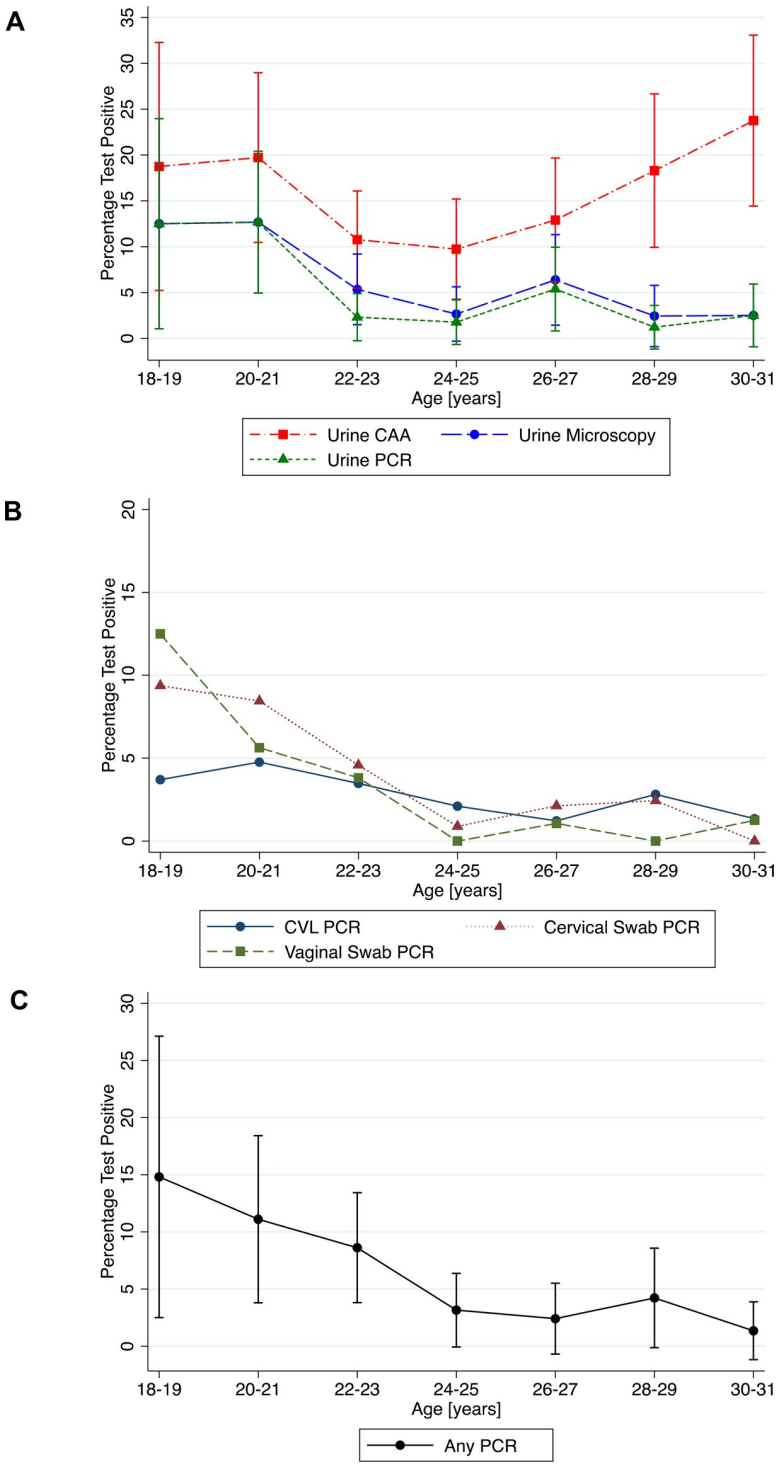

Fig 3. Distribution of positive diagnostic test results by age group. A. Distribution of positive urine CAA, urine microscopy, and urine PCR results by age group* B. Distribution of positive genital PCR results by age group* C. Distribution of any positive genital PCR result by age group* *Please see S1 Table for further details of the numbers of women tested per time point

https://doi.org/10.1371/journal.pntd.0008337.g003

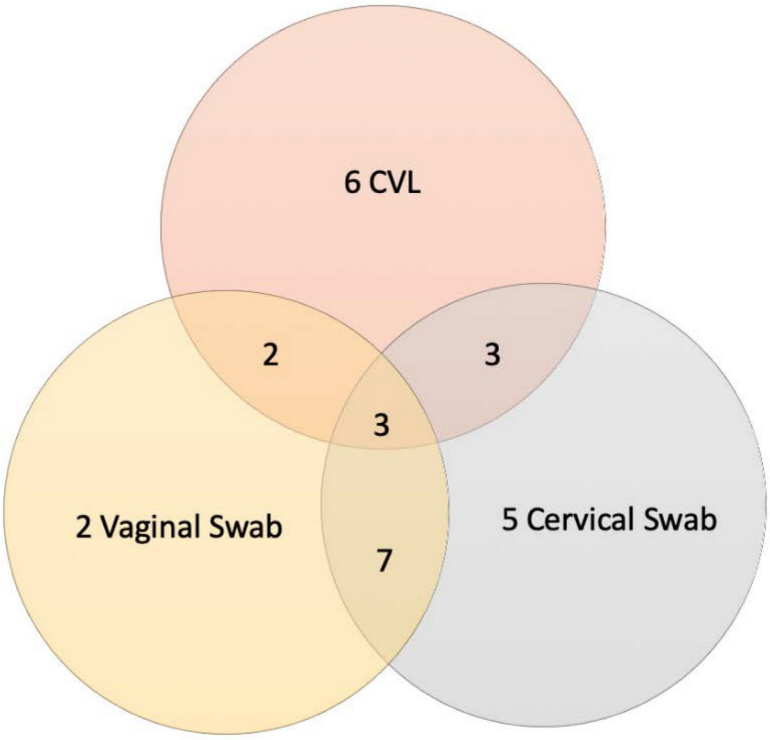

Fig 4. Venn Diagram of 28* Positive PCR-based Self-Collected Specimens (paired with CVL) for *Schistosoma* by Collection Type *2 participants with positive genital self-collected specimens did not follow up in clinic for cervicovaginal lavage.

https://doi.org/10.1371/journal.pntd.0008337.g004

Scalable, affordable, and field-acceptable diagnostics are necessary to improve our understanding of the current population prevalence of FGS and to limit the negative health impact of HIV-1, infertility, and gynaecologic symptoms. After repeated fresh-water exposures, girls living in endemic areas may develop the genital changes associated with FGS prior to sexual debut or the onset of menstruation [36]. A single dose treatment of praziquantel will decrease egg production. but will only kill a proportion of living schistosomes [3]. Data on the effect of praziquantel treatment in FGS are limited, with small numbers and heterogeneous methods, but do suggest a proportion of FGS lesions remain despite praziquantel treatment [37–39]. Thus, early identification of FGS and subsequent praziquantel treatment prior to the age of 21 is associated with beneficial outcomes, such as lower prevalence of contact bleeding, lower odds of sandy patches [40], and lower rates of sub-fertility [41]. Prevention efforts cannot be underestimated, and young women should be prioritized to obtain the maximum benefit from the roll-out of FGS diagnosis and treatment.

To date, no previous studies of FGS diagnostics have used CVL as a reference standard and cross-study comparisons are limited due to heterogeneity of methods [8, 12, 14, 21]. Previously published work has reported low sensitivity and high specificity of *Schistosoma* CVL PCR for FGS diagnosis in African populations [8, 14, 21]. In adult Zimbabwean women (ages 15–49), CVL PCR sensitivity was 53% and specificity 79% compared with cervical visualization and histopathology [14]. The study had some limitations relating to loss of specimens due to cold chain malfunctions, and extended specimen storage time (10 years), potentially affecting PCR outcomes. In an adolescent South African population (age range 15–23), CVL PCR was compared with urine microscopy, cervical visualization, and urogenital symptoms by latent class analysis with a sensitivity of 52.4% (33.2–73.6) and specificity of 42.4% (37.9–47.0) [21].

Table 3. Sensitivity and specificity of genital specimens for the detection of *Schistosoma* DNA by PCR.

Reference Standard	Index test	Sensitivity (%)	Specificity (%)
Cervicovaginal lavage PCR	Vaginal swab PCR	35.7 [5/14] (12.8–64.9)	98.2 [504/513] (96.7–99.2)
	Cervical swab PCR	42.9 [6/14] (17.7–71.1)	97.7 [501/513] (95.9–98.8)
	Vaginal or Cervical Swab PCR	57.1 [8/14] (28.9–82.3)	97.3 [499/513] (95.5–98.5)
Cervical swab PCR	Cervicovaginal Lavage PCR	33.3 [6/18] (13.3–59.0)	98.4 [501/509] (96.9–99.3)
	Vaginal Swab PCR	55.0 [11/20] (31.5–76.9)	99.3 [579/583] (98.3–99.8)
Vaginal Swab PCR	Cervicovaginal Lavage PCR	35.7 [5/14] (12.8–64.9)	98.2 [504/513] (96.7–99.2)
	Cervical Swab PCR	73.3 [11/15] (44.9–92.2)	98.5 [579/588] (97.1–99.3)
Vaginal or Cervical Swab PCR	Cervicovaginal Lavage PCR	36.4 [8/22] (17.2–59.3)	98.8 [499/505] (97.4–99.6)
Composite FGS Diagnosis*	Vaginal swab PCR	50.0 [15/30] (31.3–68.7)	100.0 [499/499] (99.3–100.0)
	Cervical swab PCR	66.7 [20/30] (47.2–82.7)	100.0 [499/499] (99.3–100.0)
	Vaginal or Cervical Swab PCR	80.0 [24/30] (61.4–92.3)	100.0 [499/499] (99.3–100.0)
	Cervicovaginal Lavage PCR	50.0 [14/28] (30.6–69.4)	100.0 [499/499] (99.3–100.0)

Abbreviations: CAA–Circulating Anodic Antigen, CS–Cervical Swab, CVL–Cervicovaginal Lavage, PCR–Polymerase Chain Reaction for the detection of *Schistosoma* DNA, VS–Vaginal Swab

*Composite FGS Diagnosis–any positive PCR result on a genital specimen (CVL, vaginal swab or cervical swab)

https://doi.org/10.1371/journal.pntd.0008337.t003

Together, these studies further illustrate the limited performance of *Schistosoma* PCR in the absence of a reliable FGS reference standard [15].

The performance of diagnostic tests for schistosome infection can vary by background parasite prevalence [42]. Important information regarding disease stage, intensity and morbidity may be missed in the absence of appropriate diagnostics [31]. No one test captures all characteristics of infection simultaneously and with perfect sensitivity. Conventional diagnostic tests (reagent strips and urine microscopy) are insensitive, especially in low-prevalence areas, where light intensity infections can be underestimated [42]. The overall prevalence of schistosome infection in our study population was 5.5% by single urine microscopy, meeting the WHO classification of a low-risk community [43]. Egg detection in urine microscopy may increase slightly with serial urine collections [7, 44]. The prevalence of FGS, as diagnosed by a positive PCR in any genital specimen, was 5.0% with site variations (2.8%-6.9%). Our study took place in two communities within Livingstone. Community-A is closer to a water source and women in Community-A had a higher overall prevalence of schistosome infection and FGS. The sensitivity of microscopy, urine PCR, and CAA diminish in areas of low parasite prevalence [42], or after treatment with praziquantel [44]. Sensitivity of CAA can be increased by using a larger sample volume [45]. Our results confirm previous findings that urine CAA has higher sensitivity than microscopy in low-endemic settings [42] and further illustrates that schistosome infection is focal and prevalence can vary within neighbouring communities.

When comparing the performance of sampling techniques for parasite DNA retrieval, cervical swabs detected 5 cases of FGS not detected by vaginal swabs and CVL. This was also true

Table 4. Sub-analysis of sensitivity and specificity of self-collected genital swabs compared with cervicovaginal lavage for the detection of *Schistosoma* DNA in participants with positive urine specimens: CAA(n = 78), microscopy (n = 32), PCR (n = 26).

Reference test	Index test	Sensitivity (%)	Specificity (%)
Urine CAA			
Cervicovaginal lavage PCR	Vaginal swab PCR	50.0 [4/8] (15.7–84.3)	90.0 [63/70] (80.5–95.9)
	Cervical swab PCR	62.5 [5/8] (24.5–91.5)	87.1 [61/70] (77.0–93.9)
	Vaginal or cervical swab PCR	87.5 [7/8] (47.3–99.7)	85.7 [60/70] (75.3–92.9)
Urine Microscopy			
Cervicovaginal lavage PCR	Vaginal swab PCR	55.6 [5/9] (21.2–86.3)	73.9 [17/23] (51.6–89.3)
	Cervical swab PCR	66.7 [6/9] (29.9–92.5)	56.5 [13/23] (34.5–76.8)
	Vaginal or cervical swab PCR	88.9 [8/9] (51.8–99.7)	56.5 [13/23] (34.5–76.8)
Urine PCR			
Cervicovaginal lavage PCR	Vaginal swab PCR	55.6 [5/9] (21.2–86.3)	70.6 [12/17] (44.0–89.7)
	Cervical swab PCR	66.7 [6/9] (29.9–92.5)	47.1 [8/17] (23.0–72.2)
	Vaginal or cervical swab PCR	88.9 [8/9] (51.8–99.7)	47.1 ([8/17] 23.0–72.2)
Active Schistosome Infection (Urine positive for PCR, CAA, or Microscopy)			
Cervicovaginal lavage PCR	Vaginal swab PCR	55.6 [5/9] (21.2–86.3)	90.3 [65/72] (81.0–96.0)
	Cervical swab PCR	66.7 [6/9] (29.9–92.5)	86.1 [62/72] (75.9–93.1)
	Vaginal or cervical swab PCR	88.9 [8/9] (51.8–99.7)	84.7 [61/72] (74.3–92.1)

https://doi.org/10.1371/journal.pntd.0008337.t004

for vaginal swabs (2 FGS cases) and CVL (6 FGS cases) that were not detected in other sites examined by PCR-based methods (Fig 4). In the absence of self-collecting cervical cytology, it is not possible to confirm if the participants self-sampled from the cervix. However, the longer swab and collection techniques likely allowed a high cervicovaginal specimen that contributed to a greater number of PCR positive samples than vaginal swabs. Studies of FGS histopathology report the most common location for egg deposition is the cervix [6], which may explain the higher proportion of positive specimens in specimens detecting DNA from this site. Genital swabs are also field-appropriate, acceptable to participants, and scalable, while CVL requires vaginal speculum insertion and is performed by trained health workers.

The BILHIV study was conducted in a low prevalence area, therefore the presented estimates of sensitivity are subject to a high degree of imprecision due to the small numbers of total FGS cases. There was excellent retention with 87.4% (527/603) of participants presenting to the clinic for CVL, with very little missing data. The women self-collected vaginal and cervical specimens privately in their own homes. This raises the question of false negative genital swabs, which could be addressed by measuring B-globin PCR as a positive control to confirm the presence of human DNA [22]. BILHIV data confirm previous findings that *Schistosoma* DNA can be detected in the genital tract in the absence of egg excretion [7, 8], and in participants with negative CAA. While these specimens may appear as false positives, the reported specificity of PCR for detecting *Schistosoma* DNA is near 100% [14, 21]. *S. haematobium* eggs

can be detected in semen [46] and our study did not objectively evaluate for PSA or other markers of recent sexual contact.

Overall, the relatively high concordance of DNA detection in genital self-collected specimens and CVL suggest that self-collection methods for the diagnosis of FGS are feasible in resource limited areas. Drawing on the experience of HPV, self-sampling has been shown to be scalable and effective in real-world settings [47]. As the focus in schistosomiasis shifts from morbidity control to elimination, data on the performance of diagnostic tests for infection and morbidity in low-prevalence settings becomes increasingly applicable [48]. To achieve elimination of both infection and chronic disease such as FGS, low-prevalence areas require novel and innovative interventions and diagnostic strategies to provide site-appropriate, accurate prevalence estimates [48].

Conclusion

Genital self-sampling increased the overall number of PCR-based FGS diagnoses in a field setting, compared with cervicovaginal lavage. Schistosomiasis is focal and background parasite prevalence may impact test sensitivity. Home-based self-sampling may represent a scalable alternative method for FGS community-based diagnosis in endemic resource limited setting.

Supporting information

S1 Text. Supplementary materials and methods.
(DOCX)

S1 Table. Positive *Schistosoma* diagnostic test results by age.
(DOCX)

Acknowledgments

We would like to thank the enthusiastic and tireless efforts of the BILHIV field workers Ethel Mwansa, Mwiingana Lukonga, Ruth Mwanza, Mervis Kantukaleza, and Judith Lungu. Our work would not have been possible without practical support from Clement Mwakamui, George Chaila, and Hilary Sipulila (Zambart). We are extremely grateful for laboratory support from Barry Kosloff, Maina Cheeba and Friday Mbesa (Zambart). We gratefully acknowledge Eric A.T. Brienen (LUMC) for performing the genital PCR analysis and Claudia J. de Dood (LUMC) and Pytsje T. Hoekstra (LUMC) for performing the CAA analysis. We thank the ODK forum for assistance with questionnaire construction and Chrissy Roberts (LSHTM) for his invaluable support with 3D printing, ODK, and other essentials.

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References

1. Lai YS, Biedermann P, Ekpo UF, Garba A, Mathieu E, Midzi N, et al. Spatial distribution of schistosomiasis and treatment needs in sub-Saharan Africa: a systematic review and geostatistical analysis. Lancet Infect Dis. 2015; 15(8):927–40. Epub 2015/05/26. https://doi.org/10.1016/S1473-3099(15)00066-3 PMID: 26004859.
2. Phiri A. "Zambia geared to eliminate bilharziasis". Ministry of Health Zambia. Available at https://www.moh.gov.zm/?p=5472. Accessed March 16, 2020.
3. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014; 383 (9936):2253–64. Epub 2014/04/05. https://doi.org/10.1016/S0140-6736(13)61949-2 PMID: 24698483; PubMed Central PMCID: PMC4672382.
4. Kjetland EF, Leutscher PD, Ndhlovu PD. A review of female genital schistosomiasis. Trends Parasitol. 2012; 28(2):58–65. Epub 2012/01/17. https://doi.org/10.1016/j.pt.2011.10.008 PMID: 22245065.
5. World Health Organization. Schistosomiasis, WHO reports substantial treatment progress for school-age children. 2017. Available at: https://www.who.int/neglected_diseases/news/WHO_schistosomiasis_reports_substantial_treatment_progress_sac/en/ [accessed April 10, 2020].
6. Mutengo M, Mwansa J, Kaonga K, Sianongo S, Wamulume H, Shinondo C. Presence of Schistosomiasis in Genital Biopsies from patients at the University Teaching Hospital in Lusaka, Zambia. Medical Journal of Zambia. 2009; 36(3):114–8.
7. Poggensee G, Kiwelu I, Saria M, Richter J, Krantz I, Feldmeier H. Schistosomiasis of the lower reproductive tract without egg excretion in urine. Am J Trop Med Hyg. 1998; 59(5):782–3. Epub 1998/12/05. https://doi.org/10.4269/ajtmh.1998.59.782 PMID: 9840597.
8. Pillay P, van Lieshout L, Taylor M, Sebitloane M, Zulu SG, Kleppa E, et al. Cervical cytology as a diagnostic tool for female genital schistosomiasis: Correlation to cervical atypia and Schistosoma polymerase chain reaction. CytoJournal. 2016; 13:10. https://doi.org/10.4103/1742-6413.180784 PMID: 27168759; PubMed Central PMCID: PMC4854169.
9. Poggensee G, Sahebali S, Van Marck E, Swai B, Krantz I, Feldmeier H. Diagnosis of genital cervical schistosomiasis: comparison of cytological, histopathological and parasitological examination. Am J Trop Med Hyg. 2001; 65(3):233–6. Epub 2001/09/20. https://doi.org/10.4269/ajtmh.2001.65.233 PMID: 11561710.
10. Kjetland EF, Ndhlovu PD, Gomo E, Mduluza T, Midzi N, Gwanzura L, et al. Association between genital schistosomiasis and HIV in rural Zimbabwean women. AIDS. 2006; 20(4):593–600. https://doi.org/10.1097/01.aids.0000210614.45212.0a PMID: 16470124.
11. Kjetland EF, Poggensee G, Helling-Giese G, Richter J, Sjaastad A, Chitsulo L, et al. Female genital schistosomiasis due to *Schistosoma haematobium*. Clinical and parasitological findings in women in rural Malawi. Acta Trop. 1996; 62(4):239–55. Epub 1996/12/30. S0001706X96000265 [pii]. https://doi.org/10.1016/s0001-706x(96)00026-5 PMID: 9028409.
12. Randrianasolo BS, Jourdan PM, Ravoniarimbinina P, Ramarokoto CE, Rakotomanana F, Ravaoalimalala VE, et al. Gynecological manifestations, histopathological findings, and schistosoma-specific polymerase chain reaction results among women with Schistosoma haematobium infection: a cross-sectional study in Madagascar. The Journal of infectious diseases. 2015; 212(2):275–84. https://doi.org/10.1093/infdis/jiv035 PMID: 25725656; PubMed Central PMCID: PMC4482143.
13. Poggensee G, Kiwelu I, Weger V, Goppner D, Diedrich T, Krantz I, et al. Female genital schistosomiasis of the lower genital tract: prevalence and disease-associated morbidity in northern Tanzania. The Journal of infectious diseases. 2000; 181(3):1210–3. https://doi.org/10.1086/315345 PMID: 10720558.
14. Kjetland EF, Ten Hove RJ, Gomo E, Midzi N, Gwanzura L, Mason P, et al. Schistosomiasis PCR in vaginal lavage as an indicator of genital Schistosoma haematobium infection in rural Zimbabwean women.

Am J Trop Med Hyg. 2009; 81(6):1050–5. https://doi.org/10.4269/ajtmh.2009.09-0081 PMID: 19996436.

15. Pillay P, Downs JA, Changalucha JM, Brienen EAT, Ramarokoto CE, Leutscher PDC, et al. Detection of Schistosoma DNA in genital specimens and urine: A comparison between five female African study populations originating from S. haematobium and/or S. mansoni endemic areas. Acta Trop. 2020; 204:105363. Epub 2020/02/09. https://doi.org/10.1016/j.actatropica.2020.105363 PMID: 32035055.

16. Kjetland EF, Kurewa EN, Mduluza T, Midzi N, Gomo E, Friis H, et al. The first community-based report on the effect of genital Schistosoma haematobium infection on female fertility. Fertil Steril. 2010; 94 (4):1551–3. Epub 2010/02/13. S0015-0282(09)04309-X [pii] https://doi.org/10.1016/j.fertnstert.2009.12.050 PMID: 20149365.

17. Helling-Giese G, Kjetland EF, Gundersen SG, Poggensee G, Richter J, Krantz I, et al. Schistosomiasis in women: manifestations in the upper reproductive tract. Acta Trop. 1996; 62(4):225–38. https://doi.org/10.1016/s0001-706x(96)00025-3 PMID: 9028408.

18. Odubamowo KH, Akinpelu OM, Lawal OO, Okolo CA, Odukogbe AA, Adekunle AO. Bilateral tubal gestation associated with schistosomiasis in an african woman. Case Rep Obstet Gynecol. 2014; 2014:674514. Epub 2015/01/13. https://doi.org/10.1155/2014/674514 PMID: 25580321; PubMed Central PMCID: PMC4279826.

19. Stothard JR, Stanton MC, Bustinduy AL, Sousa-Figueiredo JC, Van Dam GJ, Betson M, et al. Diagnostics for schistosomiasis in Africa and Arabia: a review of present options in control and future needs for elimination. Parasitology. 2014; 141(14):1947–61. Epub 2014/08/28. https://doi.org/10.1017/S0031182014001152 PMID: 25158604.

20. Downs JA, Mguta C, Kaatano GM, Mitchell KB, Bang H, Simplice H, et al. Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region. Am J Trop Med Hyg. 2011; 84(3):364–9. Epub 2011/03/03. 84/3/364 [pii] https://doi.org/10.4269/ajtmh.2011.10-0585 PMID: 21363971; PubMed Central PMCID: PMC3042809.

21. Galappaththi-Arachchige HN, Holmen S, Koukounari A, Kleppa E, Pillay P, Sebitloane M, et al. Evaluating diagnostic indicators of urogenital Schistosoma haematobium infection in young women: A cross sectional study in rural South Africa. PloS one. 2018; 13(2):e0191459. Epub 2018/02/17. https://doi.org/10.1371/journal.pone.0191459 PMID: 29451887; PubMed Central PMCID: PMC5815575.

22. Polman NJ, Ebisch RMF, Heideman DAM, Melchers WJG, Bekkers RLM, Molijn AC, et al. Performance of human papillomavirus testing on self-collected versus clinician-collected samples for the detection of cervical intraepithelial neoplasia of grade 2 or worse: a randomised, paired screen-positive, non-inferiority trial. Lancet Oncol. 2019; 20(2):229–38. Epub 2019/01/20. https://doi.org/10.1016/S1470-2045(18)30763-0 PMID: 30658933.

23. Sultana F, Mullins R, English DR, Simpson JA, Drennan KT, Heley S, et al. Women's experience with home-based self-sampling for human papillomavirus testing. BMC Cancer. 2015; 15:849. Epub 2015/11/06. https://doi.org/10.1186/s12885-015-1804-x PMID: 26536865; PubMed Central PMCID: PMC4634718.

24. Lunny C, Taylor D, Hoang L, Wong T, Gilbert M, Lester R, et al. Self-Collected versus Clinician-Collected Sampling for Chlamydia and Gonorrhea Screening: A Systemic Review and Meta-Analysis. PloS one. 2015; 10(7):e0132776. https://doi.org/10.1371/journal.pone.0132776 PMID: 26168051; PubMed Central PMCID: PMC4500554.

25. Arbyn M, Smith SB, Temin S, Sultana F, Castle P, Collaboration on S-S, et al. Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analyses. BMJ. 2018; 363:k4823. Epub 2018/12/07. https://doi.org/10.1136/bmj.k4823 PMID: 30518635; PubMed Central PMCID: PMC6278587

26. Hayes RJ, Donnell D, Floyd S, Mandla N, Bwalya J, Sabapathy K, et al. Effect of Universal Testing and Treatment on HIV Incidence—HPTN 071 (PopART). N Engl J Med. 2019; 381(3):207–18. Epub 2019/07/18. https://doi.org/10.1056/NEJMoa1814556 PMID: 31314965; PubMed Central PMCID: PMC6587177.

27. Zambian Ministry of Health. Zambia's master plan towards the elimination of Neglected Tropical Diseases, 2015–2020. In: Ministry of Community Development, p. 1–188.

28. World Health Organization 2015. Female genital schistosomiasis: a pocket atlas for clinical health-care professionals. World Health Organization. 2015. Available at, http://www.who.int/iris/handle/10665/180863 [accessed April 10, 2020].

29. Zambian Ministry of Health. Guidelines for the Etiological and Clinical Management of Sexually Transmitted Infections in Zambia, 2017.

30. Corstjens PL, De Dood CJ, Kornelis D, Fat EM, Wilson RA, Kariuki TM, et al. Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting

phosphor labels. Parasitology. 2014; 141(14):1841–55. https://doi.org/10.1017/S0031182014000626 PMID: 24932595; PubMed Central PMCID: PMC4265670.

31. van Lieshout L, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop. 2000; 77(1):69–80. https://doi.org/10.1016/s0001-706x(00)00115-7 PMID: 10996122.

32. Corstjens P, de Dood CJ, Knopp S, Clements MN, Ortu G, Umulisa I, et al. Circulating Anodic Antigen (CAA): A Highly Sensitive Diagnostic Biomarker to Detect Active Schistosoma Infections-Improvement and Use during SCORE. Am J Trop Med Hyg. 2020. Epub 2020/05/14. https://doi.org/10.4269/ajtmh.19-0819 PMID: 32400344.

33. Obeng BB, Aryeetey YA, de Dood CJ, Amoah AS, Larbi IA, Deelder AM, et al. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. Annals of tropical medicine and parasitology. 2008; 102(7):625–33. Epub 2008/09/27. https://doi.org/10.1179/136485908X337490 PMID: 18817603.

34. Pillay P, Taylor M, Zulu SG, Gundersen SG, Verweij JJ, Hoekstra P, et al. Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. Am J Trop Med Hyg. 2014; 90(3):546–52. https://doi.org/10.4269/ajtmh.13-0406 PMID: 24470560; PubMed Central PMCID: PMC3945702.

35. Balahbib A, Amarir F, Corstjens PL, de Dood CJ, van Dam GJ, Hajli A, et al. Selecting accurate post-elimination monitoring tools to prevent reemergence of urogenital schistosomiasis in Morocco: a pilot study. Infect Dis Poverty. 2017; 6(1):75. Epub 2017/04/07. https://doi.org/10.1186/s40249-017-0289-z PMID: 28381240; PubMed Central PMCID: PMC5382525.

36. Hegertun IE, Sulheim Gundersen KM, Kleppa E, Zulu SG, Gundersen SG, Taylor M, et al. S. haematobium as a common cause of genital morbidity in girls: a cross-sectional study of children in South Africa. PLoS Negl Trop Dis. 2013; 7(3):e2104. https://doi.org/10.1371/journal.pntd.0002104 PMID: 23556009; PubMed Central PMCID: PMC3605138.

37. Kjetland EF, Mduluza T, Ndhlovu PD, Gomo E, Gwanzura L, Midzi N, et al. Genital schistosomiasis in women: a clinical 12-month in vivo study following treatment with praziquantel. Trans R Soc Trop Med Hyg. 2006; 100(8):740–52. Epub 2006/01/13. S0035-9203(05)00365-2 [pii] https://doi.org/10.1016/j.trstmh.2005.09.010 PMID: 16406034.

38. Downs JA, Kabangila R, Verweij JJ, Jaka H, Peck RN, Kalluvya SE, et al. Detectable urogenital schistosome DNA and cervical abnormalities 6 months after single-dose praziquantel in women with Schistosoma haematobium infection. Trop Med Int Health. 2013; 18(9):1090–6. Epub 2013/08/14. https://doi.org/10.1111/tmi.12154 PMID: 23937701; PubMed Central PMCID: PMC4014060.

39. Richter J, Poggensee G, Kjetland EF, Helling-Giese G, Chitsulo L, Kumwenda N, et al. Reversibility of lower reproductive tract abnormalities in women with Schistosoma haematobium infection after treatment with praziquantel—an interim report. Acta Trop. 1996; 62(4):289–301. https://doi.org/10.1016/s0001-706x(96)00030-7 PMID: 9028413.

40. Kjetland EF, Ndhlovu PD, Kurewa EN, Midzi N, Gomo E, Mduluza T, et al. Prevention of gynecologic contact bleeding and genital sandy patches by childhood anti-schistosomal treatment. Am J Trop Med Hyg. 2008; 79(1):79–83. PMID: 18606767.

41. Miller-Fellows SC, Howard L, Kramer R, Hildebrand V, Furin J, Mutuku FM, et al. Cross-sectional interview study of fertility, pregnancy, and urogenital schistosomiasis in coastal Kenya: Documented treatment in childhood is associated with reduced odds of subfertility among adult women. PLoS Negl Trop Dis. 2017; 11(11):e0006101. Epub 2017/11/28. https://doi.org/10.1371/journal.pntd.0006101 PMID: 29176778; PubMed Central PMCID: PMC5720807.

42. Knopp S, Corstjens PL, Koukounari A, Cercamondi CI, Ame SM, Ali SM, et al. Sensitivity and Specificity of a Urine Circulating Anodic Antigen Test for the Diagnosis of Schistosoma haematobium in Low Endemic Settings. PLoS Negl Trop Dis. 2015; 9(5):e0003752. Epub 2015/05/15. https://doi.org/10.1371/journal.pntd.0003752 PMID: 25973845; PubMed Central PMCID: PMC4431728.

43. World Health Organization 2006. Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. Available at: https://apps.who.int/iris/bitstream/handle/10665/43545/9241547103_eng.pdf;jsessionid=564247BFBA426C9B5D72B1048671034E?sequence=1 [accessed April 10, 2020].

44. Vinkeles Melchers NV, van Dam GJ, Shaproski D, Kahama AI, Brienen EA, Vennervald BJ, et al. Diagnostic performance of Schistosoma real-time PCR in urine samples from Kenyan children infected with Schistosoma haematobium: day-to-day variation and follow-up after praziquantel treatment. PLoS Negl Trop Dis. 2014; 8(4):e2807. Epub 2014/04/20. https://doi.org/10.1371/journal.pntd.0002807 PMID: 24743389; PubMed Central PMCID: PMC3990496.

45. Corstjens PL, Nyakundi RK, de Dood CJ, Kariuki TM, Ochola EA, Karanja DM, et al. Improved sensitivity of the urine CAA lateral-flow assay for diagnosing active Schistosoma infections by using larger sample volumes. Parasites & vectors. 2015; 8:241. Epub 2015/04/22. https://doi.org/10.1186/s13071-015-0857-7 PMID: 25896512; PubMed Central PMCID: PMC4418045.

46. Leutscher PD, van Dam GT, Reimert CM, Ramarakoto CE, Deelder AM, Ornbjerg N. Eosinophil cationic protein, soluble egg antigen, circulating anodic antigen, and egg excretion in male urogenital schistosomiasis. Am J Trop Med Hyg. 2008; 79(3):422–6. PMID: 18784236.

47. Arrossi S, Paolino M, Laudi R, Gago J, Campanera A, Marin O, et al. Programmatic human papillomavirus testing in cervical cancer prevention in the Jujuy Demonstration Project in Argentina: a population-based, before-and-after retrospective cohort study. The Lancet Global health. 2019; 7(6):e772–e83. Epub 2019/05/18. https://doi.org/10.1016/S2214-109X(19)30048-8 PMID: 31097279.

48. Knopp S, Person B, Ame SM, Ali SM, Hattendorf J, Juma S, et al. Evaluation of integrated interventions layered on mass drug administration for urogenital schistosomiasis elimination: a cluster-randomised trial. The Lancet Global health. 2019; 7(8):e1118–e29. Epub 2019/07/01. https://doi.org/10.1016/S2214-109X(19)30189-5 PMID: 31255591; PubMed Central PMCID: PMC6624424.

Appendix 2 – Acceptability Manuscript

Citation: Rutty Phiri C, Sturt AS, Webb EL, et al. Acceptability and feasibility of genital self-sampling for the diagnosis of female genital schistosomiasis: a cross-sectional study in Zambia. Wellcome Open Res **2020**; 5:61.

RESEARCH ARTICLE

REVISED Acceptability and feasibility of genital self-sampling for the diagnosis of female genital schistosomiasis: a cross-sectional study in Zambia [version 2; peer review: 2 approved]

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v2 **First published:** 02 Apr 2020, **5**:61
https://doi.org/10.12688/wellcomeopenres.15482.1
Latest published: 02 Sep 2020, **5**:61
https://doi.org/10.12688/wellcomeopenres.15482.2

Abstract

Background: Female genital schistosomiasis (FGS) is a neglected and disabling gynaecological disorder that is difficult to diagnose and is part of the wider spectrum of urogenital disease caused by the waterborne parasite *Schistosoma haematobium*. Over 90% of human schistosomiasis cases are found in sub-Saharan Africa with 3.8 million people infected with schistosomes in Zambia. Reported FGS prevalence ranges from 33-75% of those with urinary schistosomiasis in endemic areas, suggesting a potentially high FGS burden in Zambia alone. The Bilharzia and HIV (BILHIV) study evaluated home self-sampling genital collection methods for the diagnosis of FGS.

Methods: Eligible participants included non-pregnant, sexually active women aged 18-31 who were previously recruited for the HPTN 071 (PopART) trial in Livingstone, Zambia. Household demographic and symptom questionnaires were administered by community workers. Participants were offered vaginal and cervical self-swabs and a urine cup. Cervicovaginal lavage (CVL) was performed in clinic by midwives. Information was collected from participants on the acceptability and feasibility of genital self-sampling.

Results: From January-August 2018, 603 women were enrolled, and 87.3% (527/603) completed clinic follow up. A high proportion of participants indicated that self-collection of specimens was “easy” or “very easy” on a 5-point Likert scale. A high proportion of women would be willing to self-collect all three specimens again in future: vaginal swab 96.7% (583/603), cervical swab 96.5% (582/603), and urine 96.2% (580/603). Overall, 90.0% (543/603) preferred to self-collect samples at home,

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 2 (revision) 02 Sep 2020	✓ report	✓ report
version 1 02 Apr 2020	? report	? report

1. **Peter Leutscher**, North Denmark Regional Hospital, Hjørring, Denmark
2. **Alexander Odaibo**, University of Ibadan, Ibadan, Nigeria

Any reports and responses or comments on the article can be found at the end of the article.

compared with sampling in the clinic Home-based self-sampling was preferred over provider-based sampling in the clinic due to greater privacy 65.0% (353/543), convenience 51.4% (279/543) and lack of needed transportation 17.7% (96/543).

Conclusions: Home based genital self-sampling for FGS diagnosis is highly acceptable. This scalable method may inform future efforts for community-based diagnosis of FGS

Keywords
female genital schistosomiasis, acceptability, feasibility, self-sampling, self-collection, vaginal self-sampling, cervical self-sampling, genital self-sampling

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Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Wellcome [205954].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Rutty Phiri C, Sturt AS, Webb EL *et al.* **Acceptability and feasibility of genital self-sampling for the diagnosis of female genital schistosomiasis: a cross-sectional study in Zambia [version 2; peer review: 2 approved]** Wellcome Open Research 2020, **5**:61 https://doi.org/10.12688/wellcomeopenres.15482.2

First published: 02 Apr 2020, **5**:61 https://doi.org/10.12688/wellcomeopenres.15482.1

REVISED **Amendments from Version 1**

We have made changes based on the suggestions made by reviewers 1 and 2. Four additional edits have been made beyond those suggested. We noted a mistake in the introductory paragraph. *S. haematobium* infection, not FGS, has been associated with HIV-1 transmission and acquisition. To clarify, we have deleted this sentence. Second, in our comparison of the online manuscript with the submitted manuscript, we noted an additional paragraph online (Methods, page 5, section "Sample collection and questionnaire"), we have added this paragraph to the current version of the manuscript. Third, the previous flow table (Figure 2) contained the wrong number of virgins (it read 17 but should be 16). Figure 2 has been updated to reflect the change. Finally, in Table 3 the proportions of women preferring testing at home versus the clinic were mislabeled. This error has now been corrected.

As suggested by reviewer 1, we have included the Likert scale in the methods section of the manuscript and clarified that the questionnaires were not performed anonymously. To improve readability, we have also reduced the number of times the denominator of n=603 has been used throughout the text.

As suggested by reviewer 2, we have included information on sample site collection (at home), also *Schistosoma* infection status of our participants in the result section and corrected some minor editorial mistakes found in the discussion section. We have removed spoken languages from Table 1.

We have also included the recently published reference to the paper of the full methods of the BILHIV study.

Any further responses from the reviewers can be found at the end of the article

Introduction

Human schistosomiasis is a waterborne parasitic disease caused by blood flukes of the genus *Schistosoma*[1,2]. It constitutes a significant public health problem causing the loss of 1,440 million years of full health worldwide, with approximately 659 million people at risk of acquiring infection[2,3]. More specifically, *Schistosoma haematobium* affects both the urinary as well as the genital tract. In female genital schistosomiasis (FGS)[1], parasite egg deposition occurs in the genital tract and it is characterized by histologic vaginal or cervical mucosal inflammation[4] and unique clinical findings[5]. FGS has been associated with infertility, a condition associated with negative social and psychological impacts in many low-income countries[6]. In addition, observational studies have suggested an association between FGS and prevalent HIV infection[7,8], and HIV transmission and acquisition[9].

Genital self-sampling has been described in the diagnosis of reproductive tract infections (RTI)[10–12] in both adults and adolescents[13] and has enhanced access to health services among hard-to-reach populations such as adolescents/young people[14], and those who do not regularly access health screening services[15,16]. A high proportion of women, including those from resource-limited settings have been found to prefer vaginal specimen self-collection[10,17] compared with clinic-based sampling. In addition to acceptability, two other factors make genital self-sampling advantageous; 1) the availability of vaginal self-sampling is effective for improving participation in specific RTI screening programmes and 2) the sensitivity of PCR-based assays on self-collected specimens compares favourably with physician-performed sampling[16,18].

The Bilharzia and HIV (BILHIV) study's primary aim was to validate home-based self-sampling for the detection of *Schistosoma* DNA with vaginal and cervical swabs against provider obtained cervicovaginal lavage in a clinic setting in an endemic area in Zambia. The BILHIV study previously found that *Schistosoma* DNA was more frequently detected in genital self-collected specimens compared to clinic-collected cervicovaginal lavage[19]. Here, we describe the acceptability and feasibility of genital self-sampling for the detection of *Schistosoma* DNA in the BILHIV study. In addition, this study also analyses the demographic predictors for participant's preference of home-based self-sampling over clinic-based sampling.

Methods

Study setting and participants

The Bilharzia and HIV (BILHIV) study was a cross-sectional study nested within two of the 12 HPTN 071 (PopART) communities in Livingstone, southern province of Zambia[20]. HPTN 071 (PopART) was a trial to measure the impact of an HIV combination prevention package, including universal test and treat[20]. Non-pregnant, sexually active women aged 18–31 who had previously been recruited for the HPTN 071 (PopART) population cohort were eligible for inclusion in BILHIV.

Sample collection and questionnaire

Between January and August 2018, specially trained population cohort research assistants visited women during the population cohort 36-month end of study follow up and enquired regarding an "expression of interest" in the BILHIV study. At a subsequent home visit, BILHIV Community Workers (BCW) evaluated study eligibility, provided participants with study information in the language of their choice, along with FGS education, and obtained written informed consent.

At the home visit, conducted in each participant's household, the BCW provided participating women with instructions for urine collection and cervical and vaginal self-swabs using educational materials including an information sheet with diagrams of the female anatomy, model vagina, and test swabs. Photos in the World Health Organization's "Female Genital Schistosomiasis Pocket Atlas" were also displayed as a visual aid. As shown in Figure 1, these educational materials were used to explain and demonstrate the procedure of self-collection of genital specimens. For swab self-collection, participants were instructed to hold a 6-inch vaginal swab (PrimeSwab, Longhorn Diagnostics, Texas, USA) at the 2 3/8-inch score mark and insert the swab vaginally until their fingers touched the labia minora. Participants moved the swab in a circular motion against the vaginal walls for a minimum of 15 repetitions. Similarly, for the cervical swab, participants were instructed to hold a 6 3/4-inch flocked swab (Miraclean, Shenzen, China) with a quadrilateral kite-shaped tip at the non-flocked end of the swab body and insert the swab vaginally until they met noticeable resistance. The participant then performed swab rotation as described above. The

Figure 1. (**A**) The Bilharzia and HIV Community Workers (BCWs) demonstrating the use of genital self-swabs by using a 3D model; (**B**) BCWs teaching by using the WHO female genital schistosomiasis atlas; (**C**) BCWs delivering questionnaires in hand-held tablets. Photo credit: A. Bustinduy; oral permission was obtained from subjects to publish these images. Images have also been edited (pixelated and cropped) to keep the identity of the subjects anonymous.

participant broke the shaft of each swab and placed the vaginal and cervical swabs in separate screw-capped microtubes (STARLAB, Hamburg, Germany). Both swab specimens and urine were placed in cool boxes for transportation to the laboratory.

Following written informed consent and specimen collection, the participants completed a non-anonymous questionnaire, with responses captured on hand-held tablets. The questionnaire assessed basic demographics, information regarding genital symptoms, sexual behaviour and also the participant's assessment of the acceptability of self-sampling, through their responses to 15 questions each measured on a five-point Likert scale (*Extended data*[21]; Table 1).

At a later date, participating women who were not currently menstruating attended Livingstone Central Hospital (LCH) cervical cancer screening clinic where a trained midwife performed a cervicovaginal lavage and images of the vagina and cervix were captured with a point-of-care colposcope (MobileODT, Tel Aviv Israel)[19]

Ethics and informed consent

All eligible participants providing written consent were recruited into the study. Participants who were unable to provide written informed consent were recruited in the presence of a witness with the participant placing their thumbprint on the consent form. The study was approved by the University of Zambia Biomedical Research Ethics Committee (reference number: 011-08-17), the Zambia National Health Research Authority and the London School of Hygiene and Tropical Medicine research ethics committee (reference number: 14506). Permission to conduct the study was given by the Livingstone District health office and the superintendent of the Livingstone Central Hospital.

Data management and statistical methods

Acceptability in our study was measured by the following outcomes: the proportion of women who rated home based self-sampling to be "*easy" or "very easy"* (for each of urine, vaginal, cervical self-sampling), the proportion who didn't experience "*pain"* while self-sampling (for each of vaginal, cervical self-sampling), the proportion who were willing to self-sample again *"in the*

Table 1. Baseline characteristics of 603 Zambian women living in *Schistosoma haematobium* endemic areas near the Zambezi river by community.

Characteristics		Overall (n=603)	Community A (n=319)	Community B (n=284)	p-value*
Age in years – Median (IQR)		24 (22-28)	26 (23-29)	24 (21-27)	<0.001
Marital Status	Single	258 (42.8%)	110 (34.5%)	148 (52.1%)	<0.001
	Married or Cohabitating	320 (53.1%)	193 (60.5%)	127 (44.7%)	
	Divorced or Separated	23 (3.8%)	15 (4.7%)	8 (2.8%)	
	Widowed	2 (0.3%)	1 (0.3%)	1 (0.4%)	
Education (highest level)	Any Primary School	167 (27.7%)	117 (36.7%)	50 (17.6%)	<0.001
	Any Secondary School	364 (60.4%)	173 (54.2%)	191 (67.3%)	
	Training in a Trade	59 (9.8%)	20 (6.3%)	39 (13.7%)	
	Degree or Higher	3 (0.5%)	3 (0.9%)	0 (0.0%)	
	None	10 (1.7%)	6 (1.9%)	4 (1.4%)	
Employment status	Working	408 (67.7%)	200 (62.7%)	208 (73.2%)	0.006
	Not Working	195 (32.3%)	119 (37.3%)	76 (26.8%)	
Current water contact	None	512 (84.9%)	263 (82.5%)	249 (87.7%)	0.02
	At Least Weekly	18 (3.0%)	11 (3.5%)	7 (2.5%)	
	Every 1–2 Months	30 (5.0%)	24 (7.5%)	6 (2.1%)	
	Every 6–12 Months	43 (7.1%)	21 (6.6%)	22 (7.8%)	
Childhood water contact	None	186 (30.9%)	96 (30.1%)	90 (31.7%)	0.22
	At Least Weekly	381 (63.2%)	208 (65.2%)	173 (60.9%)	
	Every 1–2 Months	24 (4.0%)	12 (3.8%)	12 (4.2%)	
	Every 6–12 Months	12 (2.0%)	3 (0.9%)	9 (3.2%)	
	No	572 (94.8%)	294 (92.2%)	278 (97.9%)	0.006
Self-reported history of schistosomiasis	Yes	25 (4.2%)	20 (6.3%)	5 (1.8%)	
	Maybe	6 (1.0%)	5 (1.6%)	1 (0.4%)	

*comparison of Community-A vs Community-B

future" (for each of urine, vaginal, cervical self-sampling), and the proportion who would prefer to "*sample at home"* (versus sampling in the clinic).

Participant data were entered using Open Data Kit Collect[22]. Continuous variables were summarized by mean and interquartile range (IQR), and categorical variables by frequency and percentage. Participant characteristics were compared between the two communities using Wilcoxon-Mann-Whitney, chi-squared, and Fisher's exact tests. The Mantel-Haenszel approach was used to obtain crude and age-adjusted odds ratios for the association of demographic variables with a participant's preference for home-based versus clinic-based sampling.

Results

Of 1104 women screened for BILHIV eligibility, 54.5% (603/1105) were enrolled and all completed an initial home-based visit. Of those completing the initial home visit, 87.4% (527/603) completed clinic follow up visit (Figure 2). Unless otherwise stated, the denominator for the results presented reflects the

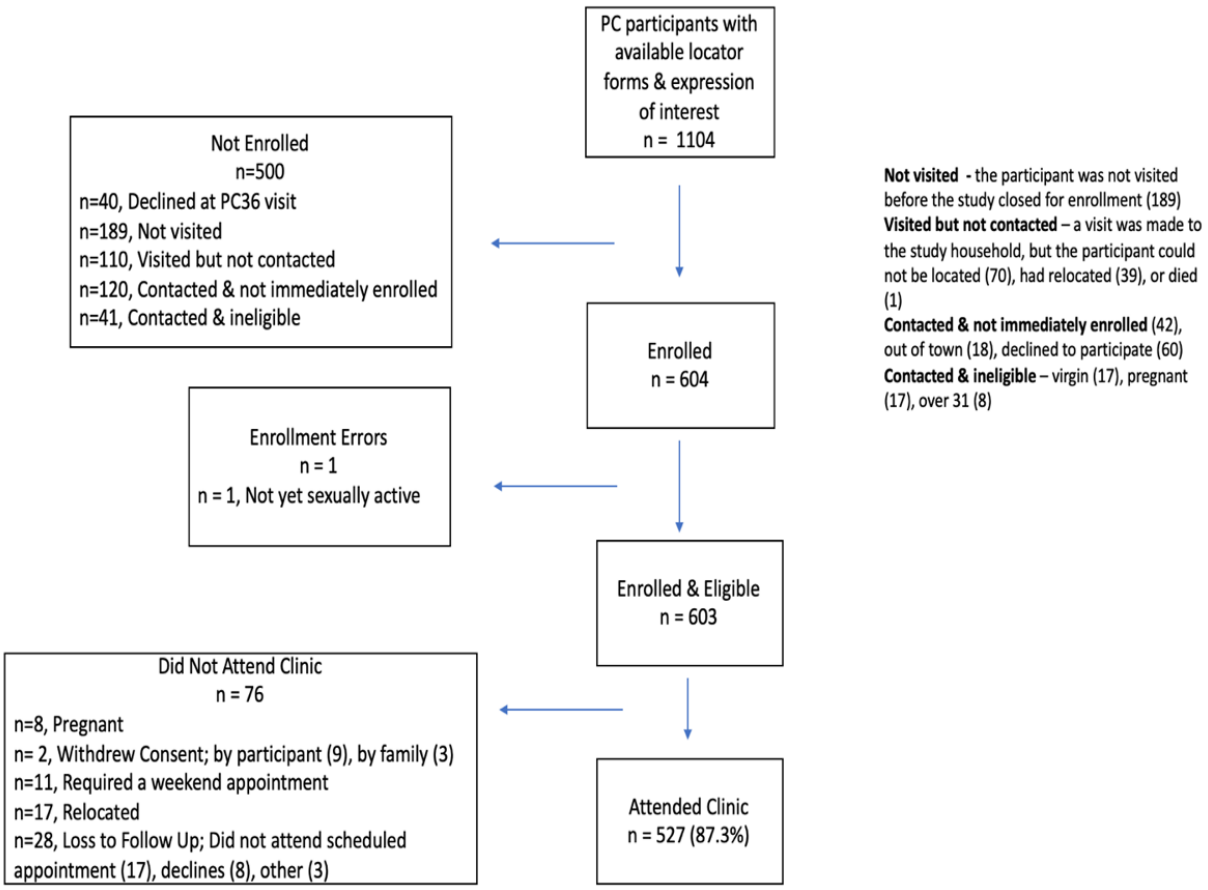

Figure 2. The Bilharzia and HIV study enrolment and sampling flow chart.

total study enrolment of 603. The median age was 24 years (IQR 22-28). More than half of participants, 60.4% (364/603), completed secondary school education and 59% (356/603) spoke primarily Nyanja (Table 1). Active schistosome infection was determined by detectable urine Circulating Anodic Antigen (CAA) (15.1%, 91/601) or microscopy (5.5%, 33/603), as previously described[18]. Compared to clinic-collected CVL (14/527, 2.7%), *Schistosoma* DNA was more frequently detected in genital self-collected specimens (24/603, 4.0%)[19].

Acceptability and feasibility

Out of 603 women recruited, a high proportion indicated that self-collection of genital specimens was "*easy*" or "*very easy*" on a 5-point Likert scale for urine collection (96.2%; 580/603), vaginal swab (94.9%; 572/603), and cervical swab (86.6%; 522/603) (Figure 3; Table 2). Most participants indicated that they would be willing to self-collect again in the future: urine 97.0% (585/603), vaginal swab 96.7% (583/603) and cervical swab 96.5% (582/603). Substantially less than half of participants reported that it was "*painful*" to self-collect vaginal specimens (3.3%; 20/603) and cervical specimens (6.8%; 41/603) (Table 2). A high proportion of women (95.7%; 577/603) indicated that they would '*recommend self-sampling to my friends*'. Overall, most women preferred to collect specimens at home (90.0%; 543/603), compared with clinic-based sampling (10.0%; 60/603), (Table 3). Women from both communities preferred to self-collect specimens from home (Community A: 89.3%, 285/319; Community B: 90.9%, 258/284; p=0.5) compared with attending the health facility. Participants preferred "*self-sampling at home*" over provider-based sampling in the clinic due to greater privacy (65.0%, 353/543), convenience (51.4%, 279/543) and lack of transportation (17.7%, 96/543) (Table 3). Participants in Community B were more confident (99.3%; 282/284) than participants in Community A (91.5%; 292/319) (p<0.001) that they collected the specimens correctly.

Overall, there was little evidence that education, marital status, community of residence, employment status, language spoken, and age were associated with a participant's preference for home-based sampling over clinic-based sampling (Table 4). Given that the preference for self-sampling was universal across the groups examined in the crude analysis, we did not undertake multivariable analysis.

Discussion

Vulnerable women and girls in sub-Saharan Africa are afflicted by FGS, a chronic gynaecologic condition. Current diagnostic strategies are limited as they rely on resources that are seldom

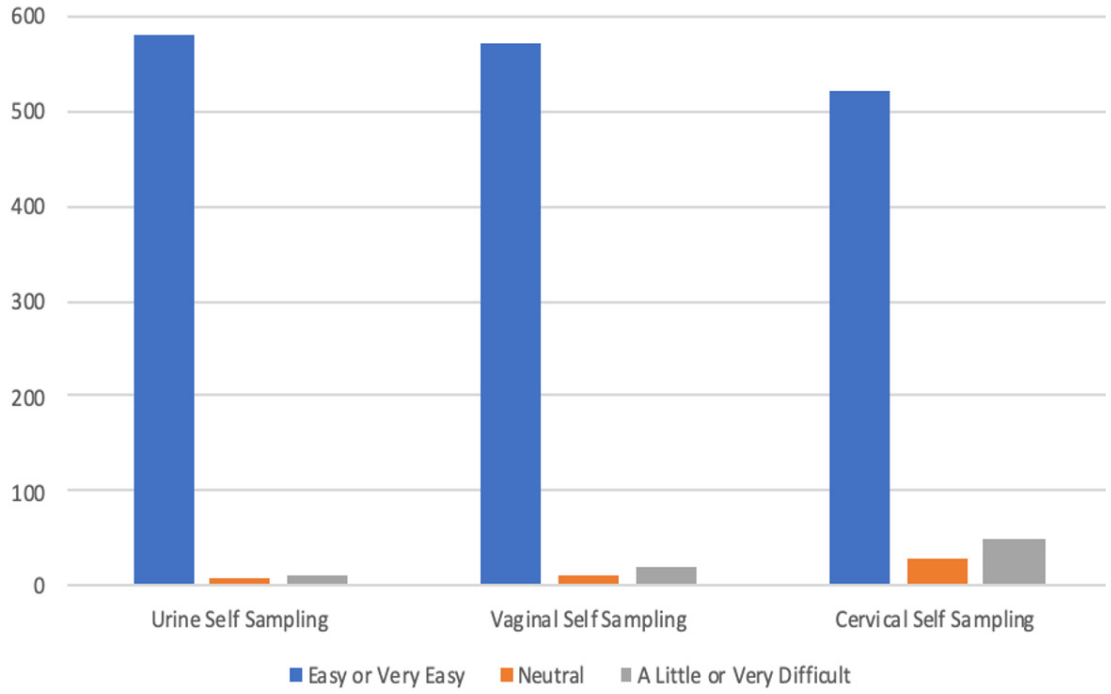

Figure 3. Ease of self-sampling in 603 Zambian women by specimen type.

Table 2. Acceptability of genital self-sampling for women from the BILHIV study (n=603).

Question	Very easy % (n)	Easy % (n)	Neutral % (n)	A little difficult % (n)	Very difficult % (n)
I found vaginal self-sampling to be	34.5 (208)	60.4 (364)	2.0 (12)	3.2 (19)	0 (0)
I found cervical self-sampling to be	26.2 (158)	60.4 (364)	5.0 (30)	8.5 (51)	0 (0)
I found collecting my own urine sample to be	56.2 (339)	40.0 (241)	1.7 (10)	2.0 (12)	0.2 (1)
Question	**Strong yes**	**Yes**	**Maybe**	**No**	**Strong no**
I would be willing to take a vaginal self-sample in the future.	42.1 (254)	54.6 (329)	2.2 (13)	1.2 (7)	0 (0)
I would be willing to take a cervical self-sample in the future.	37.0 (223)	60.0 (359)	2.5 (15)	1.0 (6)	0 (0)
I would be willing to takes a urine self-sample in the future.	38.6 (233)	58.4 (352)	2.3 (14)	0.7 (4)	0 (0)
I would recommend self-sampling to my friends.	29.0 (175)	66.7 (402)	1.8 (11)	2.0 (12)	0.5 (3)
Self-collecting a vaginal swab was painful.	0.33 (2)	3.0 (18)	3.7 (22)	77.1 (465)	15.9 (96)
Self-collecting a cervical swab was painful.	0 (0)	6.8 (41)	9.6 (58)	71.3 (430)	12.3 (74)
I am confident I collected the specimens properly.	29.0 (175)	66.2 (399)	2.7 (16)	2.2 (13)	0 (0)
I feel confident I collected a sample from my vagina.	25.7 (155)	72.3 (436)	1.3 (8)	0.7 (4)	0 (0)
I feel confident I collected a sample from my cervix.	24.5 (148)	71.6 (432)	3.5 (21)	0.3 (2)	0 (0)

Table 3. Results of the BILHIV study patient experience surveys for 603 women living in *Schistosoma haematobium* endemic areas in Livingstone, Zambia*.

Question	Participant responses	% (n)*
Do you prefer to take your samples at home, or would you prefer to take samples at the clinic?	Clinic	10.0 (60)
	Home	90.0 (543)
I prefer doing samples at home because**	It is more convenient	51.4 (279)
	I don't have transportation	17.7 (96)
	I don't have childcare	2.6 (14)
	I need to work	6.2 (34)
	I have more privacy at home	65.0 (353)
	It is easier to sample at home	66.3 (360)
	Other reason	11.4 (62)
I prefer having samples performed in clinic because**	I don't have privacy at home	26.7 (16)
	I had discomfort with collecting my own samples	13.3 (8)
	I was unsure if I did the sampling properly	30.0 (18)
	I'd like more supervision	28.3 (17)
	Other	28.3 (17)

*Proportions for home-based testing have a denominator of 543, proportions for clinic-based testing have a denominator of 60

**Participants could choose more than one answer

available in low-income settings[23]. A self-collection method that minimises reliance on health care providers would represent a scalable alternative method for FGS community-based diagnosis in endemic resource limited settings, but only if it is an acceptable procedure to perform. However, barriers to FGS diagnosis still remain, including costs, limited access to point-of-care diagnostics, and challenges with maintaining the cold chain. The cost of genital swabs (0.50$/vaginal swab and 0.30$/cervical swab) and molecular testing (6.68$/test) may be affordable in some research settings, but more field-appropriate and scalable methods should be investigated. Home based genital self-sampling for the diagnosis of FGS was highly acceptable among women aged 18 to 31 years of age enrolled in the BILHIV study in Zambia. All participating women provided all three self-collected specimens (urine, vaginal and cervical swabs), and a high proportion found vaginal self-sampling and cervical self-sampling "*easy*" or "*very easy*".

Our study is in agreement with other studies in which self-swabs were acceptable to women in geographically and ethnically diverse target populations[10,18,24]. In a study of Haitian immigrants living in the USA, the acceptability of unsupervised cervical HPV self-sampling using written instructions revealed that self-sampling was more acceptable to the majority of the women than clinician-administered sampling[24,25], and it increased screening coverage among female clinic non-attendees[15,26]. Also in an Italian study, cervical self-sampling using either a brush or a self-lavaging device was acceptable and both modalities were preferred to clinician-sampling (n=117, 68%)[27]. A systematic review on the acceptability of self-sampled screening for HPV DNA reported that self-sampling was highly acceptable among study participants in 37 studies from 24 countries across five continents[25]. Despite heterogeneity in study design, the studies in this meta-analysis suggest that self-sampling is well accepted by participants regardless of education, marital status, community of residence, employment status, language spoken, and age. Supported by these data we can conclude that our findings are likely generalizable across geographic areas and among women of varying educational background, cultures, and ethnic groups.

Substantially over half of the women in the BILHIV study reported that self-collection of specimens was "*easy" or "very*

Table 4. Factors associated with the choice of home-based sampling over clinic-based sampling, adjusted for age.

Exposure		n (home-based sampling)/N (%)	Crude OR	95% CI	aOR	95% CI	p-value
Education	None or any primary school	166/177 (94%)	reference		reference		0.31
	Any secondary school	323/364 (89%)	0.52	0.26 – 1.05	0.45	0.22 – 0.91	
	Trade training or a degree	54/62 (87%)	0.45	0.17 – 1.18	0.47	0.17 – 1.27	
Language*	Nyanja	328/356 (92%)	reference		reference		0.11
	Tonga	114/127 (90%)	0.75	0.37 – 1.50	0.75	0.38 – 1.52	
	Lozi	72/86 (84%)	0.44	0.22 – 0.88	0.44	0.22 – 0.88	
	Bemba	26/30 (87%)	0.55	0.18 – 1.71	0.55	0.18 – 1.70	
Marital status	Single	228/258 (88%)	reference		reference		0.49
	Married	292/320 (91%)	1.37	0.79 – 2.37	1.58	0.85 – 2.95	
	Divorced or widowed	23/25 (92%)	1.51	0.34 – 6.77	1.61	0.31 – 8.34	
District	Community A	285/319 (89%)	reference		reference		0.54
	Community B	258/284 (91%)	1.18	0.69 – 2.03	1.14	0.66 – 1.97	
Employment status	Not working	367/408 (90%)	reference		reference		0.91
	Working	176/195 (90%)	1.03	0.58 – 1.84	1.07	0.60 – 0.90	
Age (years)	18–22	144/158 (91%)	reference		--	--	0.62
	23–26	207/228 (91%)	0.96	0.47 – 1.95	--	--	
	27–31	192/217 (89%)	0.75	0.37 – 1.49	--	--	

easy" (urine 96.2%, vaginal swab 94.9% and cervical swab 86.6%). This is consistent with other studies that showed that study participants found genital self-sampling or the use of a self-sampling device easy to use[24,25]. The proportion with this outcome was slightly lower for cervical than vaginal sampling. Swab length and more invasive technique may account for the lower proportion of women who found cervical self-sampling "*easy*" or "*very easy*", compared with vaginal self-sampling. As another measure of acceptability, over 96% of women in the BILHIV study indicated that they were willing to self-collect *all three specimens* again in the future, which is similar to proportions reported in HPV self-collection research using cervical swabs[24,28] and curable STI research using vaginal swabs[29]. Our study, as others, further showed that a high proportion of the women indicated that they would recommend self-sampling to a friend[25]. This shows promise for the future use of peer-encouragement in the use of genital self-sampling procedures.

Our study also revealed that 90.0% of participants preferred self-sampling at home over provider-based sampling at the clinic. Our findings are similar to studies reporting a high preference for home self-sampling[25,27,28]. However, a recent meta-analysis found that the pooled estimate of women who preferred self-sampling to clinic based sampling was 59% (48 – 69%)[25]. There are some possible explanations for this. While a binary outcome was evaluated in the meta-analysis, the individual reasons for preferring home-based self-sampling to health-facility sampling vary across studies. In the BILHIV study questionnaire, the questions regarding preferences for home vs. clinic sampling included a comprehensive range of options that included 'privacy', 'convenience', 'transportation', 'work conflicts', 'no child-care', and 'ease' among others. Second, other work reports that some women preferred clinic sampling to home based self-sampling because they were not comfortable with touching their genital areas, they were unsure about the safety of self-testing, or they were concerned they would perform the test incorrectly[30].

This study benefited from HPTN 071 (PopART) because HPTN 071 (PopART) staff introduced the BILHIV study to all prospective BILHIV participants that enabled them to be familiar with the study even before it began. Further, the BILHIV study was

implemented in communities that were already familiar with the organization and the staff that worked under the HPTN 071 (PopART) study. In addition, former HPTN 071 (PopART) staff in the two study communities continued to work in the same communities under the BILHIV study. This enabled improved study performance because of the existing rapport between BILHIV staff and the community members. Standardized questionnaires were used to reduce observer bias and were performed at the time of self-sampling to minimize recall bias. However, it is important to note that the participation in the BILHIV study was limited to women who took part in the HPTN 071 (PopART) population cohort. In this scenario, bias may be related to a Hawthorne effect. This observer effect can occur as participants in a study alter their behaviour as a result of regular follow-up within a cohort[31]. The HPTN 071 (PopART) population cohort was selected through a random sampling of households and random selection of one individual within each household[31]. BILHIV study participants were selected by querying eligible members of the population cohort for an "expression of interest". There may be selection bias, in that women who expressed an interest in participating in the study may not be representative of the population as a whole and findings may not be generalizable to other sub-Saharan African communities. A larger study of genital self-sampling should be performed, preferably in areas of varied schistosomiasis endemicity.

Conclusion

We have shown high acceptability and feasibility of genital self-sampling for the diagnosis of FGS in young women (18–31 years) in a schistosomiases endemic area in Zambia. This practice has potential to increase FGS surveillance in other endemic populations. The majority of participants reported that specimen self-collection was "*easy*" or "*very easy*" with high willingness to participate in future home-based self-sampling. Results can inform future efforts for community-based diagnosis of FGS.

Data availability

Underlying data

LSHTM Data Compass: BILHIV acceptability dataset, https://doi.org/10.17037/DATA.00001618[32].

This data is under restricted access due to the assurance given to participants that responses would be kept completely confidential. This is particularly important due to the sensitivity of the data produced. The data set can be accessed by completing the Request Form, which requires that the intended use for the data is specified. Data available under the LSHTM Data Compass Data Sharing Agreement.

Extended data

Figshare: Extended data_Figshare.docx, https://doi.org/10.6084/m9.figshare.12023382.v1[21].

Acknowledgements

We wish to warmly acknowledge the BILHIV study participants, without whom this work would not have been possible.

BILHIV study team: https://www.lshtm.ac.uk/research/centres-projects-groups/bilharzia-and-hiv

References

1. WHO: **Female genital schistosomiasis: a pocket atlas for clinical health-care professionals.** World Health Organization. 2015.
Reference Source

2. Colley DG, Bustinduy AL, Secor WE, *et al.*: **Human schistosomiasis.** *Lancet.* 2014; **383**(9936): 2253–64.
PubMed Abstract | Publisher Full Text | Free Full Text

3. GBD 2017 DALYs and HALE Collaborators: **Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017.** *Lancet.* 2018; **392**(10159): 1859–922.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Jourdan PM, Holmen SD, Gundersen SG, *et al.*: **HIV target cells in Schistosoma haematobium-infected female genital mucosa.** *Am J Trop Med Hyg.* 2011; **85**(6): 1060–4.
PubMed Abstract | Publisher Full Text | Free Full Text

5. Kjetland EF, Ndhlovu PD, Mduluza T, *et al.*: **Simple clinical manifestations of genital Schistosoma haematobium infection in rural Zimbabwean women.** *Am J Trop Med Hyg.* 2005; **72**(3): 311–9.
PubMed Abstract

6. Miller-Fellows SC, Howard L, Kramer R, *et al.*: **Cross-sectional interview study of fertility, pregnancy, and urogenital schistosomiasis in coastal Kenya: Documented treatment in childhood is associated with reduced odds of subfertility among adult women.** *PLoS Negl Trop Dis.* 2017; **11**(11): e0006101.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Kjetland EF, Ndhlovu PD, Gomo E, *et al.*: **Association between genital schistosomiasis and HIV in rural Zimbabwean women.** *AIDS.* 2006; **20**(4): 593–600.
PubMed Abstract | Publisher Full Text

8. Downs JA, Mguta C, Kaatano GM, *et al.*: **Urogenital schistosomiasis in women of reproductive age in Tanzania's Lake Victoria region.** *Am J Trop Med Hyg.* 2011; **84**(3): 364–9.
PubMed Abstract | Publisher Full Text | Free Full Text

9. Wall KM, Kilembe W, Vwalika B, *et al.*: **Schistosomiasis is associated with incident HIV transmission and death in Zambia.** *PLoS Negl Trop Dis.* 2018; **12**(12): e0006902.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Arrossi S, Paolino M, Laudi R, *et al.*: **Programmatic human papillomavirus testing in cervical cancer prevention in the Jujuy Demonstration Project in Argentina: a population-based, before-and-after retrospective cohort study.** *Lancet Glob Health.* 2019; **7**(6): e772–e83.
PubMed Abstract | Publisher Full Text

11. Serwadda D, Wawer MJ, Shah KV, *et al.*: **Use of a hybrid capture assay of self-collected vaginal swabs in rural Uganda for detection of human papillomavirus.** *J Infect Dis.* 1999; **180**(4): 1316–9.
PubMed Abstract | Publisher Full Text

12. Francis SC, Mthiyane TN, Baisley K, *et al.*: **Prevalence of sexually transmitted infections among young people in South Africa: A nested survey in a health and demographic surveillance site.** *PLoS Med.* 2018; **15**(2): e1002512.
PubMed Abstract | Publisher Full Text | Free Full Text

13. Odesanmi TY, Wasti SP, Odesanmi OS, *et al.*: **Comparative effectiveness and acceptability of home-based and clinic-based sampling methods for**

sexually transmissible infections screening in females aged 14-50 years: a systematic review and meta-analysis. *Sex Health.* 2013; **10**(6): 559–69.
PubMed Abstract | Publisher Full Text

14. Bosgraaf RP, Verhoef VM, Massuger LF, *et al.*: **Comparative performance of novel self-sampling methods in detecting high-risk human papillomavirus in 30,130 women not attending cervical screening.** *Int J Cancer.* 2015; **136**(3): 646–55.
PubMed Abstract | Publisher Full Text

15. Racey CS, Withrow DR, Gesink D: **Self-collected HPV testing improves participation in cervical cancer screening: a systematic review and meta-analysis.** *Can J Public Health.* 2013; **104**(2): e159–66.
PubMed Abstract | Publisher Full Text | Free Full Text

16. Bais AG, van Kemenade FJ, Berkhof J, *et al.*: **Human papillomavirus testing on self-sampled cervicovaginal brushes: an effective alternative to protect nonresponders in cervical screening programs.** *Int J Cancer.* 2007; **120**(7): 1505–10.
PubMed Abstract | Publisher Full Text

17. Bhatla N, Dar L, Patro AR, *et al.*: **Can human papillomavirus DNA testing of self-collected vaginal samples compare with physician-collected cervical samples and cytology for cervical cancer screening in developing countries?** *Cancer Epidemiol.* 2009; **33**(6) :446–50.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Arbyn M, Smith SB, Temin S, *et al.*: **Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analyses.** *BMJ.* 2018; **363**: k4823.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Sturt AS, Phiri CR, Webb EL, *et al.*: **Genital self-sampling compared with cervicovaginal lavage for the diagnosis of female genital schistosomiasis in Zambian women: The BILHIV study.** *PLoS Negl Trop Dis.* 2020; **14**(7): e0008337.
PubMed Abstract | Publisher Full Text | Free Full Text

20. Hayes RJ, Donnell D, Floyd S, *et al.*: **Effect of Universal Testing and Treatment on HIV Incidence - HPTN 071 (PopART).** *N Engl J Med.* 2019; **381**(3): 207–18.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Phiri CR, Sturt A, Webb EL, *et al.*: **Extended data_Figshare.docx**. *figshare*.
http://www.doi.org/10.6084/m9.figshare.12023382.v1

22. Hartung C: **Open Data Kit Tools to Build Information Services for Developing Regions**. *ICTD 2010 Proceedings of the 4th ACM/IEEE International Conference on Information and Communication Technologies and Development*. [accessed May 28, 2019].
Reference Source

23. O'Brien DP, Ford N, Djirmay AG, *et al.*: **Female Genital Schistosomiasis and HIV: Research Urgently Needed to Improve Understanding of the Health Impacts of This Important Coinfection.** *J Acquir Immune Defic Syndr.* 2019; **80**(5): 489–93.
PubMed Abstract | Publisher Full Text

24. Barbee L, Kobetz E, Menard J, *et al.*: **Assessing the acceptability of self-sampling for HPV among Haitian immigrant women: CBPR in action.** *Cancer Causes Control.* 2010; **21**(3): 421–31.
PubMed Abstract | Publisher Full Text

25. Nelson EJ, Maynard BR, Loux T, *et al.*: **The acceptability of self-sampled screening for HPV DNA: a systematic review and meta-analysis.** *Sex Transm Infect.* 2017; **93**(1): 56–61.
PubMed Abstract | Publisher Full Text

26. Gok M, Heideman DA, van Kemenade FJ, *et al.*: **Offering self-sampling for human papillomavirus testing to non-attendees of the cervical screening programme: Characteristics of the responders.** *Eur J Cancer.* 2012; **48**(12): 1799–808.
PubMed Abstract | Publisher Full Text

27. Igidbashian S, Boveri S, Spolti N, *et al.*: **Self-collected human papillomavirus testing acceptability: comparison of two self-sampling modalities.** *J Womens Health (Larchmt).* 2011; **20**(3): 397–402.
PubMed Abstract | Publisher Full Text

28. Gottschlich A, Rivera-Andrade A, Grajeda E, *et al.*: **Acceptability of Human Papillomavirus Self-Sampling for Cervical Cancer Screening in an Indigenous Community in Guatemala.** *J Glob Oncol.* 2017; **3**(5): 444–54.
PubMed Abstract | Publisher Full Text | Free Full Text

29. Jones HE, Altini L, de Kock A, *et al.*: **Home-based versus clinic-based self-sampling and testing for sexually transmitted infections in Gugulethu, South Africa: randomised controlled trial.** *Sex Transm Infect.* 2007; **83**(7): 552–7.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Tisci S, Shen YH, Fife D, *et al.*: **Patient acceptance of self-sampling for human papillomavirus in rural china.** *J Low Genit Tract Dis.* 2003; **7**(2): 107–16.
PubMed Abstract | Publisher Full Text

31. Hayes R, Ayles H, Beyers N, *et al.*: **HPTN 071 (PopART): rationale and design of a cluster-randomised trial of the population impact of an HIV combination prevention intervention including universal testing and treatment - a study protocol for a cluster randomised trial.** *Trials.* 2014; **15**: 57.
PubMed Abstract | Publisher Full Text | Free Full Text

32. Sturt A, Bustinduy A, Phiri C: **BILHIV acceptability dataset. [Data Collection].** London School of Hygiene & Tropical Medicine, London, United Kingdom. 2020.
http://www.doi.org/10.17037/DATA.00001618

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 15 October 2020

https://doi.org/10.21956/wellcomeopenres.17751.r40248

✔ **Peter Leutscher**
Center for Clinical Research, North Denmark Regional Hospital, Hjørring, Denmark

The manuscript is now approved from my part. Congratulations to the team.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 09 September 2020

https://doi.org/10.21956/wellcomeopenres.17751.r40247

✔ **Alexander Odaibo**
Parasitology Research Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria

The authors have clarified and addressed the reservations I had in my first review.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology and control of schistosomiasis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 20 July 2020

https://doi.org/10.21956/wellcomeopenres.16935.r39268

?

Alexander Odaibo
Parasitology Research Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria

The authors intended to evaluate the acceptability and feasibility of genital self-sampling for female genital schistosomiasis in a given cohort in Zambia. The intention is good and the execution is appropriate but there are a few clarifications to be made.

Methods:

1. No information was provided on the number of participants recruited for the study.

2. It is not clear from the article if there was a common sample collection centre for all participants or sample collection was done at the residence of each participant.

3. What was the *Schistosoma haematobium* infection status of the participants at the time of study?

Results:

1. Figure 2 is superfluous and what is the relevance of the table under result?

2. No information on how the home-based sampling by the women compared with the clinic sampling done in this study, instead readers are referred a paper that is still under review.

Discussion:

1. The authors may need to rephrase the first sentence under discussion to avoid starting the sentence with an abbreviation (FGS).

2. **Schistosomiases** should be changed to **schistisomiasis**.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Epidemiology and control of schistosomiasis.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Aug 2020

Amaya Bustinduy, London School of Hygiene & Tropical Medicine, London, UK

We thank Prof Odalbo for his helpful comments. To respond to his queries, this is a point by point response.

- No information was provided on the number of participants recruited for the study.

We apologize for any confusion, the information about total number of participants recruited can be found in the "Abstract" and also on page 6 under "Results" in the 1st and 3rd sentences in our final submitted manuscript. For convenience, we have highlighted this in the accompanying manuscript.

- It is not clear from the article if there was a common sample collection centre for all participants or sample collection was done at the residence of each participant.

Thank you for this input. We have added your point regarding sample collection was done on method section, sub section "Sample Collection and Questionnaire" line 6.

- What was the Schistosoma haematobium infection status of the participants at the time of study?

In the "Results" section we have now included the schistosome infection status of the participants in the study. We used both urine microscopy and Circulating Anodic Antigen (CAA). As the CAA is not species-specific, we have expressed infection status as "active schistosome infection".

Results:

- Figure 2 is superfluous and what is the relevance of the table under result?

Thank you for your review. Figure 2 is the BILHIV Study Flow Diagram. We thought providing this information to readers might provide transparency regarding the included participants and enhance interpretation of the study's generalisability.

In terms of the tables in the "Results" section:
Table 1 describes the baseline characteristics of 603 study participants

Table 2 give more information regarding experiences women had during self-sampling. Table 3 describes the results of the patient experience surveys for 603 study participants Table 4 describes the demographic factors associated with the choice of home-based sampling over clinic-based sampling We feel these tables should be maintained.

- No information on how the home-based sampling by the women compared with the clinic sampling done in this study, instead readers are referred a paper that is still under review.

Thank you very much for bring up this point and for any inconvenience. In the third paragraph of the "Introduction" we describe that "the BILHIV study found that *Schistosoma* DNA was more frequently detected in genital self-collected specimens compared to clinic-collected cervicovaginal lavage". At the time this manuscript was originally submitted we had not yet published the main results for the BILHIV study, however the paper that was under review is now published and it is cited as reference number 19.

Discussion:

- The authors may need to rephrase the first sentence under discussion to avoid starting the sentence with an abbreviation (FGS).

Thank you for this input. We have rephrased the sentence starting with abbreviation FGS under "Discussion", line 1.

- Schistosomiases should be changed to schistisomiasis.

Thank you for your observation. We have changed 'Schistosomiases to schistosomiasis' under "Conclusion" in line 2.

Competing Interests: No competing interests were disclosed.

Reviewer Report 03 July 2020

https://doi.org/10.21956/wellcomeopenres.16935.r38334

?

Peter Leutscher
Center for Clinical Research, North Denmark Regional Hospital, Hjørring, Denmark

In this manuscript, results from questionnaire in a urogenital self-sampling study are presented addressing schistosomiasis infection in women aged 18-31 living in Zambia.
Overall, the conclusion is clear. Self-sampling is very well accepted by the women, and apparently with only minor discomfort/distress, if any. This approach of home sampling as an alternative to urogenital sampling in a clinical setting seems attractive by providing the women elements of individual convenience and privacy.

However, as also presented in the discussion, the study findings may not necessarily translate to the same extent into other communities in sub-Saharan Africa, mainly because a bias being

potentially implicated due to previous study activities taking place in the populations before the self-sampling study. Therefore, another study should be performed, and preferable in different schistosomiasis endemic communities in Southern Africa to control for the potential bias.

Other comments:

- Inclusion criteria (e.g age, non-pregnancy) are not stated in Methods.
- Why inform about a pending paper by Stuart *et al.*? More interesting to know about a probably pending paper presenting the lab findings (SH DNA).
- A 5-point Likert scale has been used. This information should be presented in the Methods and not in the Results.
- No information whether the questionnaire was performed anonymously or not, apparently not if one looks at the field image. Then there would have been a unique opportunity to uncover various reasons for reported lack of confidence, acceptance, comfort etc – even only reported in a minority of the women.
- Is information about the different dialects in Table 1 of interest for the reader?
- Interesting that 32.3% of women are given the status as “Not working”.
- Information about Childhood water contact seems not that relevant (recall-bias) in adult women.
- How has self-reported history of schistosomiasis been assessed?

Results

- N=603 stated 17 times. Should be adequate to mention once, the number of study participants.
- Confusing that the percentages in Table 3 (I don’t have transportation, I have more privacy at home) are different from those in the main text (17.7% vs 15.9%; 65.0% vs. 58.5).

Discussion

- *FGS is a chronic gynaegological condition that afflicts vulnerable women*… What is meant by “vulnerable”?
- *Current diagnostic strategies are limited as they rely on resources that are seldom available in low-income settings. S*elf-sampling does not contribute significantly to solving this problem, only to minor extent, having the women to perform the sampling themselves instead of a health care provider (e.g a nurse). Other major cost will remain, including lab technicians, equipment, reagents etc.
- Participants should always be acknowledged.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 20 Aug 2020

Amaya Bustinduy, London School of Hygiene & Tropical Medicine, London, UK

In this manuscript, results from questionnaire in a urogenital self-sampling study are presented addressing schistosomiasis infection in women aged 18-31 living in Zambia. Overall, the conclusion is clear. Self-sampling is very well accepted by the women, and apparently with only minor discomfort/distress, if any. This approach of home sampling as an alternative to urogenital sampling in a clinical setting seems attractive by providing the women elements of individual convenience and privacy.

However, as also presented in the discussion, the study findings may not necessarily translate to the same extent into other communities in sub-Saharan Africa, mainly because a bias being potentially implicated due to previous study activities taking place in the populations before the self-sampling study. Therefore, another study should be performed, and preferable in different schistosomiasis endemic communities in Southern Africa to control for the potential bias.

Thank you for this input. We have added your point regarding performing other studies in schistosomiasis endemic communities in different regions to the discussion. Lines 67-70 under discussion.

Inclusion criteria (e.g age, non-pregnancy) are not stated in Methods.

We apologize for any confusion. In the BILHIV study, women were eligible if they were sexually active aged 18-31 who were not pregnant and had previously been recruited

for the HPTN 071 (PopART) population cohort were eligible for inclusion in BILHIV. In the on-line version (https://wellcomeopenresearch.org/articles/5-61) the inclusion criteria are stated in the first sentence of the abstract methods. In the main manuscript, the inclusion criteria are stated in the Methods section in the second sentence methods under "study setting and participants". In the tracked-changes manuscript we have highlighted these areas for clarity.

Why inform about a pending paper by Stuart et al.? More interesting to know about a probably pending paper presenting the lab findings (SH DNA).

We agree that the section in question should be modified. In the section "Sample collection and questionnaire" in the final sentence, we have removed the wording "Sturt, A *et al.* paper under review" and instead we reference the BILHIV study manuscript. As you have suggested, this manuscript provides the full laboratory results.

A 5-point Likert scale has been used. This information should be presented in the Methods and not in the Results.

We apologize for any confusion, the information about the Likert scale was included in the methods section in our final submitted manuscript, but it seems not to have been uploaded into the online manuscript. We have highlighted this in the accompanying manuscript.

No information whether the questionnaire was performed anonymously or not, apparently not if one looks at the field image. Then there would have been a unique opportunity to uncover various reasons for reported lack of confidence, acceptance, comfort etc – even only reported in a minority of the women.

You are correct that the questionnaire was not performed anonymously. We have clarified this in the methods.

Is information about the different dialects in Table 1 of interest for the reader?

Thank you for this input, although we agree with the reviewer that dialects are not directly of importance to the study outcome, we would like to keep them in the table as they contribute to a more holistic appreciation of the study participants and their background. This may be of interest for certain readers.

Interesting that 32.3% of women are given the status as "Not working".

We agree that this is interesting. The women were asked the question "are you currently working?" with yes/no answer. These data reflect their self-reported response to this question.

Information about Childhood water contact seems not that relevant (recall-bias) in adult women.

We agree that the variable regarding childhood water contact is subject to recall bias. However, we thought this information would provide the reader with information regarding the participant's perceived level of exposure.

How has self-reported history of schistosomiasis been assessed?

The self-reported history of schistosomiasis was not further assessed beyond the participant's self-report.

RESULTS

N=603 stated 17 times. Should be adequate to mention once, the number of study participants.

Thank you for this input. We have repeated the total enrollment number to clarify the denominator for many of the presented proportions.

Confusing that the percentages in Table 3 (I don't have transportation, I have more privacy at home) are different from those in the main text (17.7% vs 15.9%; 65.0% vs. 58.5).

Thank you for catching this! Outcomes for this variable should be divided by the proportion of women in the sampling category (prefer to sample at home, n=543). In the abstract and the main text, these proportions were mistakenly reported out of 603. The correct proportions are reported in Table 3. This has now been updated in the manuscript.

DISCUSSION

FGS is a chronic gynaegological condition that afflicts vulnerable women... What is meant by "vulnerable"?

In the setting of environmental health emergencies, the WHO describes vulnerability as "the degree to which a population, individual, or organization is unable to anticipate, cope with, resist, or recover from the impact of disasters". We feel this vulnerability also describes well the plight of women in sub-Saharan Africa in relationship to FGS, as an underreported neglected ailment. Thus, we suggest that the term could be maintained.

*Current diagnostic strategies are limited as they rely on resources that are seldom available in low-income settings. S*elf-sampling does not contribute significantly to solving this problem, only to minor extent, having the women to perform the sampling themselves instead of a health care provider (e.g a nurse). Other major cost will remain, including lab technicians, equipment, reagents etc.

Thank you for this input. We agree that many of the other costs will remain and we have attended to this in the manuscript discussion. However a full cost-effective

analysis was beyond this pilot work.

Participants should always be acknowledged.

Thank you for bringing this oversight to our attention. We have acknowledged the participants.

Competing Interests: No competing interests were disclosed.

Appendix 3 – LSHTM Occupational Health Surveillance Assessment

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

The ROYAL MARSDEN NHS Foundation Trust

OCCUPATIONAL HEALTH DEPARTMENT

Health Surveillance Assessment

Name of employee	Amy Sturt
Department/Unit	CRD/ITD
Line Manager /Supervisor	Amaya Bustinduy

Category 2	Yes
Pathogen	Schistosoma Haematobium
*Blood sample taken	Not required

*Where a baseline blood sample has been taken prior to work commencing, which will be stored at LSHTM, a second sample will be needed at the end of the period of employment at LSHTM.

Animal worker	No
Respiratory health surveillance	No

Working with material of human origin.	Yes

The above named employee is:

FIT FOR WORK

Signed: Penny Strover

Date: 04/07/2019

Appendix 4 – LSHTM CL1/2 Summary Protocol and Risk Assessment

CL1/2 Summary Protocol and Risk Assessment

Faculty of Infection and Tropical Disease

Note: *Principal Investigator takes ownership of this document and is responsible for sending a COMPLETE signed copy to Health and Safety Department*

Protocol Number	*BILHIV_CVL03*
Protocol Title	MAGPIX Assays Using Human Cervicovaginal Lavage
Organism	*Schistosoma haematobium*
Route(s) of Infection	Cercariae penetrate transdermally after hatching from a fresh-water snail. This pathogen is not transmitted human – to human. The infectious stage of this organism (cercariae) will not be present in this work. There may be presence of *S. haematobium* ova in the CVL, but this is unlikely.
Laboratory	Lab 234 Inner lab (234d) containing a category 2 hood and microcentrifuge
Project Supervisor	Amaya Bustinduy
Disinfectants	10% chloros for solid and liquid waste that has been in contact with CVL. The hood will be used for specimen opening and centrifugation. Further work will be performed on the bench. A specific area will be set aside for specimen handling. This area will be lined with Benchkote, shiny (non-absorbent) side down, which will be removed after use. Surfaces will be cleaned with 70% ethanol.
Waste Disposal	Liquid waste will be decontaminated overnight in 10% hypochlorite solution. Solid waste will be autoclaved.

Written by:	Amy Sturt			
Date:	June 14, 2019			
I, the undersigned, have reviewed the assessment of the work titled above, and declare that any risks will be controlled by the methods stated on this form and that the work will be carried out in accordance with laboratory protocols.				
Approved by: Faculty Safety Supervisor	signature redacted			
Date Approved:	26Jun2019			
Is work carried out in BSF?	☐	Yes	☒	No
Approved by: BSF Manager				
Approved by: Principal Investigator	signature redacted			
Date Approved:	27Jun2019			
This assessment should be reviewed within 3 years of approval date, or sooner in the event of a change in procedure or an incident occurs.				
Date received by H&S Department:				

To be read in conjunction with the appropriate Laboratory and Specific Code of Practices

Summary of Revisions
No previous versions

I have read and understood this Risk Assessment and will use the control methods identified.		
Name	**Signature**	**Date**

LABORATORY TECHNIQUE

Give a brief description of the technique to be used

In the MAGPIX system, beads pass into an imaging chamber containing a magnet. The beads sit in a monolayer on the magnet. LEDs excite the beads and the flourescence is detected by a CCD camera. This method is used to detect biomarkers in body fluids.

RISKS/HAZARDS IDENTIFIED:

Please list the hazards / risks of this procedure. e.g., use of sharps, use of poisons.

EMD Millipore Proprietary Reagents

- Sodium azide or proclin has been added to some reagents (L-SAPE 3-11, L-WB, MXHSM, MHX1060-1-3, MSH6060-1/2, MXH8060-1/2) as a preservative. Dispose of unused contents and waste in accordance with local regulations. Other agents can cause eye or skin irritation.

Sodium Azide

- Sodium azide is used as a preservative in Merck Millipore's Human Cytokine Standard. This standard is not used neat. It is diluted by five to six serial five-fold dilutions and 25 microliters is added to 5 wells in the 96 well plate.
- Sodium azide is highly toxic if swallowed.
- Potential risk through inhalation of dust and aerosols.
- Contact with acids liberates toxic gas, but no acids are used in this protocol.
- Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Mini Centrifuge

- Risk of shock from electrical appliance.
- Imbalanced load leading to rotator damage and possible ejection of debris from centrifuge.

MAGPIX Microspheres

- MAGPIX microbeads are damaging to the environment.

Cervicovaginal lavage that may be infected with human pathogens

- Risk of infection (e.g. hepatitis B) when working with unscreened CVL.

Bio-Rad Magnetic Plate Washer

- The handheld washer used to wash to wash plates during the MAGPIX assay has minimal magnetic shielding.
- Dangerous to pacemaker wearers.

Vortex

- Spills and splash injuries may occur.

Additional Hazards Identified:
No sharps will be utilized in this work

CONTROL MEASURES IN PLACE:

Please describe the control measures you will use to minimise the risks/hazards identified above.

General

- Wear lab coat and gloves throughout the procedure.

EMD Millipore Proprietary Reagents

- Sodium azide in very low concentrations may be poured down the drain followed by large amounts of water to prevent any appreciable build-up of azide metal.

Sodium Azide

- Read MSDS prior to use
- Long-sleeved fastened laboratory coat and gloves to be worn.
- Safety goggles to be worn where splashing is possible.
- Sodium azide in very low concentrations may be poured down the drain followed by large amounts of water to prevent any appreciable build-up of azide metal.

Mini Centrifuge

- Centrifuge must be PAT tested and carry a certified sticker.
- All users should be instructed on the safe use of the equipment.
- Always use rotator lid and balance odd samples with a counter weight, i.e. a similar tube containing water.

MAGPIX Microspheres

- Do not dispose of beads down the sink. The beads should be filtered out of solution though a sintered funnel unit and disposed of as hazardous waste.
- Used plates should be discarded in the autoclave bin

CVL

- All biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of potentially infectious agents. Please see CVL02 CVL Risk Assessment for additional details.
- Samples will be centrifuged and aliquoted prior to use in the MAGPIX machine to limit exposure to *S. haematobium* ova and other potentially infectious agents as detailed in CVL02. Centrifugation and specimen lid opening will be performed in a safety hood.
- Wear lab coat, gloves, mask and safety glasses when using unscreened biological materials.

- Never work with your own CVL.
- Ensure that your Hepatitis B vaccination is up to date. Students and other staff who do not have a Hepatitis B vaccination should be made aware that all human bodily fluids should be considered infectious and should follow all other recommendations within this risk assessment.
- All staff using this pathogen should report to Occupational Health prior to commencing work.
- Liquid and solid waste will be decontaminated overnight in 10% hypochlorite solution.

Magnetic Plate Washer

- Pacemaker wearers should use an automated plate washer with a magnetic plate.
- Set the plate shaker at a speed that will provide the maximum orbital mixing without splashing liquid outside of the plate wells (setting 5-7 or 500-800 rpm).
- Wear eye protection given the risk of splashing with potentially infectious fluids.

Vortex

- Use an appropriate vessel for the sample (screw cap rings are preferable)
- Ensure the cap or lid is on tight before vortexing.
- Grasp the tube with a paper towel, and vortex the tube perpendicularly
- To avoid spills, wait 1-2 minutes before opening the vessel after vortexing.

Summary of Procedures & Precautions

Procedure	Risks and Control Measures
Protocol – SOP if appropriate 1. See the risk assessment entitled "Handling Previously Collected Human Cervicovaginal Lavage (CVL)" for further details regarding specimen opening, centrifugation and transfer to the bench. 2. Add 200 uL of wash buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature 3. Decant Wash Buffer and remove residual amount from all wells by inverting plate and tapping it smartly onto absorbent towels several times.	**Risks Identified:** Wash buffer can be irritating to skin **Control Measure:** wear PPE (including gloves) at all times **Risk Identified:** Plate shakers can splash liquid. **Control Measure:** Set the plate shaker at a speed that will provide the maximum orbital mixing without splashing liquid outside of the plate wells (setting 5-7 or 500-800 rpm).

	Wear PPE (gloves, lab coat, face and eye mask) at all times.
4. Add 25uL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background). 5. Add 25 uL of Assay Buffer to the sample wells. 6. Add another 25uL of Assay Buffer to the background, standards, and control wells (in place of the serum matrix). 7. Add 50uL of CVL into the appropriate wells 8. Vortex mixing bottle and add 25uL of mixed beads to each well (during addition of beads, shake bottle intermittently to avoid settling). 9. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker for 2 hours at room temperature. 10. Gently remove well contents and wash plates 2 times. 11. **WASH STEP:** Rest plate on handheld magnet for 60 seconds. Remove well contents by decanting the plate in an appropriate waste receptacle. Gently tap on absorbent pad to remove residual liquid. Wash plate with 200uL Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds. Repeat. 12. Add 25 uL of Detection Antibodies into each well	**Risks Identified:** Human Cytokine/Chemokine Standard contains low concentrations of sodium azide but can be harmful if swallowed and can cause eye damage. **Control Measure:** Wear PPE (gloves, lab coat, face and eye mask) at all times. Use appropriate disposal techniques to avoid reactions of high concentrations with lead and copper plumbing **Risk Identified:** Some wells will contain small amounts of sodium azide and other skin/eye irritants. **Control Measure:** Wear PPE (gloves, lab coat, face and eye mask) at all times. Collect in appropriate waste receptable to ensure appropriate disposal techniques

13. Seal, cover with foil, and incubate with agitation on a plate shaker for 1 hour at room temperature. 14. Add 25uL of Streptavidin-Phycoerythrin to each well containing the 25uL of Detection Antibodies 15. Seal, cover with foil, and incubate with agitation on a plate shaker for 30 minutes at room temperature. 16. Gently remove well contents and wash plates 2 times (see step 11 above). 17. Add 150uL of Drive Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes. 18. Run plates on MAGPIX.	**Risk Identified:** Human cytokine detection antibodies can cause eye irritation. **Control Measure:** Wear PPE (gloves, lab coat, face and eye mask) at all times. **Risk Identified:** Streptavidin-Phycoerythrin can cause eye irritation. **Control Measure:** Wear PPE (gloves, lab coat, face and eye mask) at all times.
<u>Decontamination/Fumigation</u>	10% chloros for solid and liquid waste that has been in contact with CVL. The hood will be used for specimen opening and centrifugation. Further work will be performed on the bench. A specific area will be set aside for specimen handling. This area will be lined with Benchkote, shiny (non-absorbent) side down, which will be removed after use. Surfaces will be cleaned with 70% ethanol.

BSF Procedures & Precautions

If work is carried out in BSF, please complete table below

Risks	Control Measures
Allergen exposure:	
Needlestick/Sharps injury:	

Transport:	
Pathogen exposure:	
Other Considerations (e.g. bites, chemicals	

Appendix 5 – University of Zambia biomedical research committee ethical approval
Parent study, reference 011-08-17

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm
Assurance No. FWA00000338
IRB00001131 of IORG0000774

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

26th October, 2017.

Your Ref: 011-08-17.

Dr. Amaya Bustinduy,
London School of Hygiene & Tropical Medicine,
C/o School of Public Health,
P.O Box 50110,
Lusaka.

Dear Dr. Bustinduy,

RE: RESUBMITTED RESEARCH PROPOSAL: "VALIDATION OF HOME-BASED SAMPLING AND VAGINAL SELF-SAMPLING FOR THE DIAGNOSIS OF FEMALE GENITAL SCHISTOSOMIASIS (FGS) IN ZAMBIAN WOMEN WITH AND WITHOUT HIV SEROCONVERSION" (REF. No. 011-08-17)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 20th October, 2017. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- Where appropriate, apply in writing to National Health Research Authority for permission before you embark on the study.
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

Dr. S.H Nzala
VICE-CHAIRPERSON

Date of approval: 26th October, 2017. **Date of expiry**: 25th October, 2018.

Appendix 6 – University of Zambia biomedical research committee ethical approval
Sub-studies amendment, reference 011-08-17

UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
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Fax: + 260-1-250753
Federal Assurance No. FWA00000338

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
E-mail: unzarec@unza.zm
IRB00001131 of IORG0000774

2nd August, 2019.

Your Ref: 011-08-17.

Dr. Amaya Bustinduy,
ZAMBART,
P.O Box 50697,
Lusaka.

Dear Dr. Bustinduy,

RE: **AMENDMENTS TO: "VALIDATION OF HOME BASED SAMPLING AND VAGINAL SELF SAMPLING FOR THE DIAGNOSIS OF FAMALE GENITAL SCHISTOSOMIASIS (FGS) IN ZAMBIAN WOMEN WITH AND WITHOUT HIV SEROCONVERSION" (REF. No. 011-08-17)**

We acknowledge receipt of your request for amendments to the aforementioned protocol in which you are proposing to investigate the association of cervicovaginal bacteria (in the protocol referred to as "microbiota") and other sexually transmitted infections with FGS.

The changes were reviewed and approved as follows:

1. More information has been added to the Secondary Objectives and to the Outcome Measures.
2. More information has been added to the literature review.
3. More information has been added to the methodology in regards to the hospital visits and to the Genital samples.
4. More information has been added to the assessment and follow up.

Yours sincerely,

Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON
Tel: +26099925304
E-Mail: s.munsaka@unza.zm

Appendix 7 – LSHTM ethical approval
Parent study, reference 14506

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

Observational / Interventions Research Ethics Committee

Dr. Amaya Bustinduy
Associate Professor in Tropical Paediatrics
Department of Clinical Research (CRD)
Infectious and Tropical Diseases (ITD)
LSHTM

19 December 2017

Dear Amaya

Study Title: Validation of home-based cervical and vaginal self-sampling for the diagnosis of Female Genital Schistosomiasis (FGS) in Zambian women with & without HIV seroconversion_revised

LSHTM Ethics Ref: 14506

Thank you for responding to the Interventions Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Protocol / Proposal	HPTN 071 schisto follow up	25/07/2017	1.0
Sponsor Letter	Bustinduy_QA1032_sponsor_27072017	27/07/2017	1.0
Safety Information	K161871.Letter.FDAApproval	14/09/2017	1.0
Investigator CV	7.6 ABustinduy-CV_July_2017	14/09/2017	1.0
Investigator CV	7.6 Sturt_CV_2016_11_15 LSHTM	14/09/2017	1.0
Information Sheet	BILHIV Nyanja201708	14/09/2017	1.0
Information Sheet	BILHIV Bemba2017	14/09/2017	1.0
Information Sheet	BILHIV Tonga201708	14/09/2017	1.0
Advertisements	BILHIV Study Flyer 1.4 UNZA BREC	16/10/2017	1.4
Information Sheet	BILHIV Consent 1.4 UNZA BREC	17/10/2017	1.4
Information Sheet	BILHIV Information Sheet 1.4 UNZA BREC	17/10/2017	1.4
Protocol / Proposal	UNZA BREC Protocol BILHIV V1.4 LSHTM	17/10/2017	1.4
Protocol / Proposal	BILHIV Questionnaire 1.4 UNZA BREC	17/10/2017	1.4
Local Approval	2017.20.10 UNZABREC Approval Letter	20/10/2017	1.0
Other	2017.29.10 Sturt GCP Certificate (R2)	29/10/2017	1.0
Other	GCP_GCP Certificate (R2)	14/11/2017	1.0
Covering Letter	LSHTM Cover Letter BILHIV2v3	23/11/2017	2.3

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review

using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 8 – LSHTM ethical approval
Sub-studies, reference 14655

London School of Hygiene & Tropical Medicine
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United Kingdom
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www.lshtm.ac.uk

Observational / Interventions Research Ethics Committee

Dr Amy Sturt
LSHTM

28 June 2018

Dear Amy ,

Study Title: Evaluation of Inflammation, the Vaginal Microbiota and STI in Zambian Women with and without Female Genital Schistosomiasis.

LSHTM ethics ref: 14655

Thank you for your application for the above research, which has now been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Local Approval	2017.19.12 LSHTM 14506 Approval	19/12/2017	1.0
Consent form	BILHIV Consent V1.4	22/01/2018	1.4
Investigator CV	CV_Piet_Cools_english_feb2018	28/02/2018	1.0
Local Approval	UNZA BREC ETHICS APPROVAL AMENDMENT_March 2018	22/03/2018	1.0
Local Approval	2018.03.23 14506 LSHTM Amendment Decision Letter	23/03/2018	1.1
Investigator CV	ABustinduy-CV_April_2018	30/04/2018	1.0
Investigator CV	CV_SCF_20180405	04/05/2018	1.0
Investigator CV	2018.05.18 Sturt CV	18/05/2018	1.0
Protocol / Proposal	2018.05.22 Microbiota Protocol	22/05/2018	1.0
Local Approval	2017.20.10 UNZABREC Approval Letter	20/10/2018	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter

Chair

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Appendix 9 – Associated workshop, meeting, and conference presentations

American Society of Tropical Medicine and Hygiene National Meeting (69th), November 17-21, 2020. Toronto, Ontario, Canada. Oral Abstract #1565. "**The role of vaginal inflammation in HIV-1 vulnerability in Zambian women with Female genital schistosomiasis"**.

London School of Hygiene and Tropical Medicine. Research Degree Student's Poster Day – March 2020. "**The role of vaginal inflammation in HIV-1 vulnerability in Zambian women with female genital schistosomiasis"**.

London School of Hygiene and Tropical Medicine. International Statistics and Epidemiology Monthly Meeting, January 19, 2021. London, United Kingdom. **"Genital immune activation in Zambian women with female genital schistosomiasis".**

London School of Hygiene and Tropical Medicine. Clinical Research Department Laboratory Group Monthly Meeting, March 16, 2021. London, United Kingdom. **"Associations of female genital schistosomiasis with the cervicovaginal microbiota and reproductive tract infections in Zambian women".**