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

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16
17 **Running Head:** FGS and HIV-1 Incidence
18 **Word Count:** 3376/3500
19
20 **Key Points:** FGS has been associated with prevalent HIV-1. In this study, women with FGS had
21 higher rates of HIV-1 seroconversion, however there was no statistical evidence of an
22 association.
23

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38 ***Background:***

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

41 ***Methods:***

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

48 ***Results:***

49 Incident HIV-1 infections were observed in 4.1% (20/492) participants. Women with FGS were
50 twice as likely to seroconvert as women without FGS but with no statistical evidence for a
51 difference (aRR 2.16, 95%CI[0.21–12.30], p=0.33). Exploratory analysis suggested an association
52 with HIV-1 acquisition among women with ≥2 positive genital PCR specimens (RR 6.02, [0.58–
53 34.96]), p=0.13).

54 ***Conclusions:***

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

58

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

61 **Introduction:**

62 In 2019, an estimated 56 million women were living with female genital schistosomiasis (FGS), a
63 neglected tropical disease that results when eggs from the parasite *Schistosoma (S.) haematobium*
64 are deposited in reproductive tract tissues [1]. Tissue-entrapped eggs incite a cellular response [2],
65 ultimately resulting in FGS-related morbidity, including infertility [3], and distinct cervicovaginal
66 manifestations [4, 5]. In sub-Saharan Africa, there is a geographical association between areas of
67 high *S. haematobium* prevalence and HIV-1 infection [6], and FGS has been associated with
68 prevalent HIV-1 [7] with biological plausibility for a causal relationship [3, 8]. Despite global
69 advances in HIV-1 treatment and prevention, gender related disparities still exist, with particularly
70 heightened risk among young women aged 15-24 years [9]. HIV-1 vulnerability in young women
71 is multifactorial, including biological, behavioural, demographic, social, and structural
72 components [10]. The potential role of FGS as an underreported and preventable co-factor in HIV-
73 1 vulnerability needs further investigation.

74

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

84 universal evaluation for genital involvement) and prevalent HIV-1 have been mixed, with evidence
85 of an association with prevalent HIV-1 in a study of Tanzanian women [13], some evidence of an
86 association with prevalent HIV-1 in a study of Zimbabwean women [14], but with no evidence of
87 an association with prevalent HIV-1 in men and women with urinary *S. haematobium* in Congo
88 [15]. While *S. haematobium* seropositivity in women has been associated with HIV-1 acquisition
89 [16], the association of FGS with incident HIV-1 has not been described.
90
91 FGS diagnosis is challenging and its burden is likely underreported. The presence of parasite eggs
92 or DNA in cervicovaginal tissue is diagnostic of FGS, [17, 18], and, historically, biopsy is used as
93 a reference standard [4, 19]. However, theoretical concerns regarding post-biopsy HIV-1
94 acquisition has limited its acceptance in research settings [17]. Polymerase chain reaction (PCR)
95 on cervicovaginal lavage (CVL) is a less-invasive means of FGS diagnosis, albeit with imperfect
96 sensitivity [17, 18]. Well-defined clinical manifestations have been associated with FGS [5, 20]
97 but are variably correlated with the presence of *S. haematobium* eggs [19, 20] or DNA [17] in
98 genital tissue. The identification of clinical lesions with colposcopy is observer-dependent and
99 subject to low specificity [21]. Homogeneous yellow sandy patches have been associated with
100 herpes simplex virus-2 and human papillomavirus, and abnormal blood vessels may be associated
101 with cervical intraepithelial neoplasia [5]. Urine microscopy and circulating anodic antigen (CAA)
102 can be used to detect active schistosome infection [18, 22], but do not assess involvement of genital
103 tissue. While either *S. haematobium* and *S. mansoni* can cause FGS, the majority of cases are
104 attributed to *S. haematobium* [23, 24] and the current study focuses on *S. haematobium*. We
105 conducted an array of diagnostic tests for *S. haematobium* infection (CAA and urine microscopy)
106 and FGS (portable colposcopy, cervical swabs, vaginal swabs, and cervicovaginal lavage), and

107 have previously demonstrated that self-collected genital swabs had comparable sensitivity to
108 clinic-based, midwife-collected CVL for the detection of *Schistosoma* DNA by real-time PCR
109 [18].
110
111 The longitudinal follow-up of women in the HPTN 071 (PopART) trial in two schistosomiasis-
112 endemic communities in Zambia provided an opportunity for a nested study exploring the
113 association of FGS with HIV-1 incidence.
114
115 **Methods**
116 ***Study setting and participants***
117 The cross-sectional bilharzia and HIV (BILHIV) study was nested in HPTN 071 (PopART), a
118 cluster randomized trial assessing the impact of an HIV-1 combination prevention package
119 including "universal testing and treatment" [25]. HIV-1 incidence was measured in a Population
120 Cohort (PC) comprised of one randomly selected adult (18 to 44 years of age) from a random
121 sample of households in each community who provided data and blood samples at baseline, 12,
122 24, and 36 months [25]. Between January and August 2018, after the 36-month HPTN 071
123 (PopART) PC visit, trained community workers conducted home visits to women who had
124 expressed interest in the BILHIV study [18]. Women in Livingstone, Zambia were eligible if they
125 were 18-31 years old, not pregnant, sexually active, and resident in one of the two urban *S.*
126 *haematobium* endemic communities that participated in one of two HPTN 071 (PopART)
127 intervention arms.
128

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

151 described [18, 30]. Analysing the equivalent of 417 μL urine, a CAA value of >0.6 pg/mL was
152 considered positive [22].
153

154 ***PCR for detection of Schistosoma* DNA**

155 DNA extraction and PCR was performed at LUMC as previously described, using a custom
156 automated liquid handling station (Hamilton, Switzerland) [20, 31]. DNA was extracted from
157 200μL of specimen (cervical swab, vaginal swab, CVL) with QIAamp spin columns (QIAGEN
158 Benelux; Venlo, The Netherlands). Detection of the schistosome-specific internal-transcribed-
159 spacer-2 (ITS2) target was performed by real-time PCR as previously described [18, 31]. This
160 PCR does not differentiate between *Schistosoma* species. DNA amplification and detection were
161 performed with the CFX96 Real Time PCR Detection System (BioRad, California, USA). The
162 output in threshold cycles (Ct), reflecting the parasite-specific DNA load in the tested sample, was
163 analysed using BioRad CFX software. Parasite DNA loads were categorized by the following pre-
164 specified C_t thresholds: high (C_t<30), moderate (30≤C_t<35), low (35≤ C_t <50) and negative (no C_t
165 detected), as previously described [32].
166

167 ***Patient Consent Statement***

168 The study was approved by the University of Zambia Biomedical Research Ethics Committee
169 (reference 011-08-17), the Zambia National Health Research Authority and the London School of
170 Hygiene and Tropical Medicine Ethics Committee (reference 14506). Permission to conduct the
171 study was given by Livingstone District Health Office and the Livingstone Central Hospital
172 superintendent. Each participant provided written informed consent.
173

174 ***FGS Definitions***

175 Comparison groups were defined by the results of four investigations: genital PCR, colposcopy
176 image review, urine CAA, and urine microscopy. Participants were grouped by the outcomes of
177 their diagnostic tests into three mutually exclusive categories. *FGS* was defined as at least one
178 positive genital PCR (cervical swab, vaginal swab or CVL) (Figure 1). *Probable/possible FGS*
179 was defined as the presence of either a positive urine diagnostic (CAA or microscopy) or one of
180 four cervicovaginal manifestations suggestive of FGS on portable colposcopy, or both, with
181 negative genital PCR (Figure 1). FGS *negative* was defined as negative results on all diagnostics.
182

183 **Statistical Methods**

184 Characteristics of study participants were summarized by frequency and percentage. Women
185 living with HIV-1 (WLHIV) at HPTN 071 (PopART) baseline were excluded from further
186 analyses. HIV-1 incidence was calculated as the number of seroconversions per 1000 person-years
187 of follow up. Participants contributed person-time for the calculation of HIV-1 incidence starting
188 with their first HIV-1 test and ending at date of HIV-1 seroconversion for those who seroconverted,
189 or at the date of last follow-up or the end of scheduled follow-up (whichever occurred earliest) for
190 women who did not seroconvert. HIV-1 seroconversion was assumed to occur at the midpoint
191 between the last negative and the first positive HIV-1 test. We assumed that FGS acquisition
192 occurred prior to HPTN 071 (PopART) enrolment [33]. BILHIV study participants were
193 consecutively recruited from the PC, providing the opportunity to compare the rate of incident
194 HIV-1 infection in women with and without FGS, with power determined by the number of HIV-
195 1 seroconversions and FGS prevalence. Data on HIV-1 outcomes were not available until after
196 BILHIV study closure.

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

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

212 **Results**

213 A total of 603 eligible women from the HPTN 071 Population Cohort were enrolled in the BILHIV
214 study. WLHIV at HPTN 071 (PopART) trial entry (n=107, 17.7%) were excluded, with 492
215 (82.1%) included in this analysis (Figure 2). Of the included participants, 14% (69/492) did not
216 attend clinic for CVL.

217

218 ***Baseline characteristics***

219 The majority of participants had received at least secondary education, were not working, and
220 reported being currently sexually active. A small proportion of women reported current water
221 contact, but more than half reported childhood water contact. Active schistosome infection,
222 defined as either a positive urine microscopy (5.5%, 27/492) or detectable CAA (15.1%, 74/492),
223 was detected in 15.7% (77/492) of participants.
224

225 ***HIV incidence***

226 The 492 women without HIV-1 at HPTN 071 (PopART) study entry provided a total of 1,164
227 person-years of follow-up, during which time, 20 (4.1%) incident HIV-1 infections were
228 measured, for an overall rate of 17.2 (95% CI 11.1–26.6) seroconversions per 1000 person-years.
229 HIV-1 incidence rates are shown by baseline characteristics in Table 1.
230

231 HIV-1 incidence rates were 23.6 (14.2 – 39.2) in women aged 18-24 years compared with 9.5 (3.9–
232 22.7) in women aged 25-31, (RR 0.40 [0.15–1.10]), p=0.06 (Table 1)). The HIV-1 seroconversion
233 rate decreased as the household size increased (p=0.007, test for trend) and increased as the number
234 of lifetime sexual partners increased (p=0.01, test for trend). Women self-reporting a history of
235 STI were more likely to seroconvert than women without self-reported STI (RR 5.76 [1.92–17.22],
236 p=0.009) (Table 1). No other sociodemographic or behavioural characteristics were associated
237 with HIV-1 incidence. After adjusting for age, there remained strong evidence that a higher number
238 of people residing in a household (p=0.008, test for trend), a higher number of lifetime sexual
239 partners (p=0.01, test for trend), and self-reported history of STI (aRR 6.05 [2.02–18.12], p=0.008)
240 were associated with HIV-1 seroconversion. Additionally, there was no evidence for an association

241 between urinary schistosome infection (as defined by urine CAA and/or microscopy) and HIV-1
242 seroconversion (Table 2).
243

244 ***Association between FGS and HIV-1 seroconversion***

245 FGS was identified in 5.3% of women (26/492), defined as any positive genital PCR (cervical
246 swab 3.5%, [17/492]; vaginal swab 2.4%, [12/492]; or CVL 3.1%, [13/423]). Among women with
247 a negative genital PCR, results from both urine and colposcopy imaging were positive in 4.5%
248 (21/466) of participants and results from either urine or colposcopy imaging were positive in
249 31.1% (145/466). Of the participants with *probable/possible FGS*, 63.8% (106/166) had
250 colposcopy changes in isolation (Figure 1), of whom 62.3% (66/106) had abnormal blood vessels
251 and 37.7% (40/106) had grainy or homogenous yellow sandy patches on colposcopy. There were
252 218 (44.3%) participants who were negative on all diagnostic tests. The rate of HIV-1
253 seroconversion (per 1000 person/year) in women with FGS (31.0 [7.8 – 123.9]) was higher than
254 in the FGS *negative* group (11.3[5.1–25.1]) (Table 2) but without statistical evidence of a
255 difference between these rates in either univariable or multivariable analyses (crude RR 2.75
256 [0.27–15.36], p=0.26; aRR 2.16 (0.21–12.30), p=0.33) (Table 2).
257

258 ***Exploratory analyses:* Schistosoma *DNA concentrations and disease burden***

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

264 these groups, n=9 of the women overlapped and the same two participants contributed
265 seroconversions in both groups. There were no HIV-1 seroconversions in participants with one
266 positive genital PCR.
267
268 **Discussion**
269 This study is the first to examine the association of PCR-defined FGS with HIV-1 incidence. We
270 found that women with FGS were twice as likely to seroconvert than women in the comparison
271 group albeit with wide confidence intervals and no statistical evidence for a difference. While
272 barriers to implementation still exist, PCR for FGS diagnosis is reproducible, has high specificity,
273 and can be performed on self-collected genital specimens [18].
274
275 While some cross-sectional studies show an association between schistosomiasis and prevalent
276 HIV-1 infection [13, 23], this association is not universally reported [34, 35]. The association
277 between schistosomiasis and HIV-1 is complex and cross-study comparisons require the
278 consideration of many aspects, including schistosome species (*S. haematobium* versus *S. mansoni*),
279 diagnostic tests used, assessment of genital involvement, and presentation of subgroup analyses
280 e.g. by participant's sex. Our findings, while limited by power, show a point estimate consistent
281 with increased risk of incident HIV-1, but with a wide confidence interval. Recently, two case-
282 control studies nested within longitudinal African cohorts have retrospectively assessed the
283 association between schistosome infection status and HIV-1 seroconversion with conflicting
284 results [16, 36]. A Zambian study showed an increased risk of HIV-1 acquisition in *S.*
285 *haematobium* antibody positive women (aHR=1.4, $p<0.05$), but not men [16]. Similar to our
286 results, the study from Kenya and Uganda did not show an association between active schistosome

287 infection and HIV-1 seroconversion, including in subgroup analyses by sex, schistosome species,
288 and infection intensity [36]. Notably, however, neither of these nested case-control studies
289 evaluated genital infection status. FGS may enhance HIV-1 vulnerability, with proposed
290 mechanisms including cervicovaginal barrier dysfunction [37], local recruitment or activation of
291 HIV-1 target cells [3], and *Schistosoma*-related alterations in integrin [38] or co-receptor [39]
292 expression.
293
294 Schistosomiasis and FGS are preventable and current WHO control measures recommend
295 praziquantel preventive chemotherapy [40]. However, current control programmes do not
296 universally achieve 75% coverage of school-aged children, representing substantial missed
297 opportunities for prevention [41, 42]. The 2025 AIDS targets place communities at risk for HIV-
298 1 in the centre of societal, system, and service enablers with a call for between sector integration
299 and synergy to advance the HIV-1 response [43]. Programmatic synergy including integrated
300 sexual and reproductive health programmes could leverage and scale-up existing HIV-1 treatment
301 and prevention resources to include FGS screening and treatment programs.
302
303 We carried out two ad hoc exploratory analyses. The intensity of schistosome infection, defined
304 by serum CAA concentration, has been strongly correlated with HIV-1 prevalence [13]. Thus, first,
305 we investigated whether *Schistosoma* DNA concentrations might be associated with HIV-1
306 infection in an analysis of 13 participants with FGS and moderate/high *Schistosoma* DNA
307 concentrations. We found no evidence of an association between FGS and HIV-1 acquisition,
308 albeit with wide confidence intervals. Additionally, participants with moderate to high seminal
309 egg excretion have higher seminal cytokine concentrations than S. haematobium negative

310 participants (Leutscher 2005). Thus secondly, we investigated the association between multiple
311 positive genital PCR specimens as a potential proxy marker of higher FGS burden and HIV-1
312 seroconversion in 13 women with ≥2 positive genital specimens for *Schistosoma* DNA. We found
313 weak evidence of an association between FGS and HIV-1 acquisition, which was less pronounced
314 in the age-adjusted estimates. These findings are hypothesis generating for the association between
315 FGS cervicovaginal disease burden or *Schistosoma* DNA concentrations and HIV-1.
316
317 This study was nested within a large population-based HIV-1 prevention trial and is the first
318 prospective study to document FGS in Zambia, but also had some relevant limitations. Similar to
319 other FGS studies using imperfect available diagnostics, there is the risk of potential diagnostic
320 misclassification, especially in these low-prevalence and low-intensity study settings. We defined
321 FGS by PCR positivity based on its semi-quantitative nature and precedent in FGS diagnosis [17,
322 18, 44]. However, *S. haematobium* eggs in semen from a male sex partner could potentially be
323 detected by PCR of vaginal specimens. We were unable to adjust for potential confounders beyond
324 age and are thus unable to exclude unmeasured and residual confounding. This was related to the
325 low number of HIV-1 seroconversions and FGS cases, which also resulted in a loss of power.
326 Overall, the effect sizes suggest the possibility of a relationship we were not sufficiently powered
327 to detect. While the prevalence of HIV-1 in the study population was high at 17.9%, the two
328 participating communities were enrolled in HPTN 071 (PopART) as intervention sites, potentially
329 reducing the number of HIV-1 seroconversions [25]. The prevalence of urinary *S. haematobium*
330 infection in this study was 5.5% (27/492), lower than anticipated, defined by the World Health
331 Organization as a low prevalence area [45]. In addition, while schistosomiasis is endemic in all of
332 Zambia's 10 provinces [46], and can be found in urban locations , it is generally considered to be

333 a focal disease of rural areas [47], and our study sites were in lower prevalence areas (<10%
334 prevalence) as defined by WHO (cite PC manual). Indeed, a 2013 survey done by the Zambian
335 Ministry of Health reported a wide range of egg-patent infection prevalence in different areas
336 ranging from 3.3 – 73.3% (median 15.0%, mean 23.3%) in school-aged children in Livingstone,
337 highlighting its focal distribution.[18]. For all the above reasons, the presented estimates, obtained
338 in a peri-urban setting, are subject to a high degree of imprecision and may not be generalizable to
339 rural communities. Lastly, vaginal and cervical swabs were self-collected by participants, raising
340 the potential for false negative genital swabs. In future work, this could be addressed by measuring
341 β-globin PCR as a positive control to confirm the presence of human DNA [48]. This study was
342 developed based on a conceptual framework which describes a potentially causal relationship
343 between FGS and HIV-1, with FGS as a potentially preventable and modifiable risk factor. In the
344 literature, albeit in cross-sectional studies, there is evidence for biological plausibility [3, 8, 12]
345 and large effect sizes for the association [7] between FGS and HIV-1. One of our study limitations
346 was the temporality of HIV-1 and FGS diagnostics. HIV-1 seroconversion was measured in HPTN
347 071 (PopART) up to three years prior to participant enrolment in the study and subsequent FGS
348 diagnosis. This sequencing assumes that FGS status and demographic descriptors at the time of
349 genital PCR sampling are similar to those at the time of HPTN 071 (PopART) study entry and/or
350 HIV-1 seroconversion. This assumption is reasonable given that FGS is thought to develop after
351 childhood water exposure [33] and persist into adulthood with chronic genital lesions, often
352 persisting despite treatment with praziquantel [49]. A large, prospective, longitudinal study in
353 areas of higher *S. haematobium* endemicity is needed to evaluate incident HIV-1 infection in
354 women with known *S. haematobium* and FGS status at study baseline. In future work, it will be

355 important to continue to analyse HIV-1 outcomes by both *S. haematobium* infection status and
356 FGS definition (PCR versus clinical findings) to evaluate HIV-1 risk profiles.
357
358 In conclusion, this analysis from a limited number of co-infected women does not show evidence
359 of association between FGS and HIV-1 incidence. The hypothesis generating observations that
360 FGS and in particular higher FGS cervicovaginal disease burden or *Schistosoma* DNA
361 concentrations may be associated with HIV-1 acquisition should be investigated in a larger
362 longitudinal study in a high FGS prevalence area to better explore the role of FGS in HIV-1
363 acquisition.

364
365
366

367 **Footnotes**

368 **Conflict of Interest Statement:**

369 The authors report no conflicts of interest

370

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421

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563

564

565 **Table 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)	

566 $^{\phi}$ test for trend p-value
567 **Any sexual activity in the last 6 months
568 †Participants who responded with "no answer" (n=2) are not shown in the table (HIV seroconversions =0)
569 †† STI history was self-reported, participants who responded with "no answer" (n=1) are not shown (HIV seroconversions =0)
570 †††Participants who responded with "no answer" (n=8) are not shown in the table (HIV seroconversions =0)
571
572

573 **Table 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)	

574

575 [**]82 participants who were negative on all diagnostic tests but missing portable colposcopy images were excluded from this analysis

576 [*]Adjusted for age

577 [#]Test for trend p-value (RR per unit of the exposure variable [FGS *Negative*, *Probable/Possible* FGS and FGS] treated as a continuous
578 variable)
579 [##]n=13 excluded (one genital PCR specimen positive)
580 [†]n=13 excluded (Ct>35)
581 [††] n=492, unless otherwise specified
582 [‡]n=490, 2 vials arrived at the laboratory empty, HIV-1 seroconversion occurred in (n=1) of these participants
583 [‡‡]defined as detectable urine CAA or positive urine microscopy
584
585
586 **Abbreviations:** aRR – adjusted rate ratio, CAA – circulating anodic antigen, Ct – cycle threshold, FGS – female genital schistosomiasis, IRR –
587 incidence rate ratio, PCR – polymerase chain reaction, PY – person-years
588

589 **FIGURE TITLES & LEGENDS**

590 **Figure 1 Title: Female Genital Schistosomiasis categories and Venn diagram illustrating**

591 **results by diagnostic test type**

592 **Figure 1 Legend:**

593 **A**. Female Genital Schistosomiasis diagnostic categories

594 **B.** Participants in the diagnostic categories by test result

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

596

597 **Figure 2 Title: Study Flow Diagram**

598 **Figure 2 Legend:**

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

600 **Visited but not contacted (n=110)**– a visit was made to the study household, but the participant

601 could not be located (70), had relocated (39), or died (1)

602 **Contacted & not immediately enrolled (n=120)– visited but not recruited** (42), out of town

603 (18), declined to participate (60)

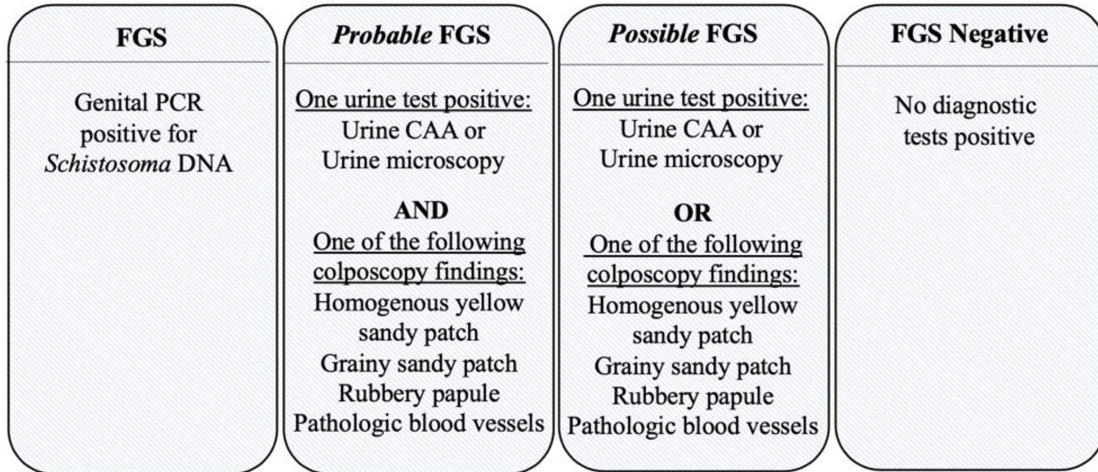
604 **Contacted & ineligible (n=41)**– virgin (16), pregnant (17), over 31 (8)

605

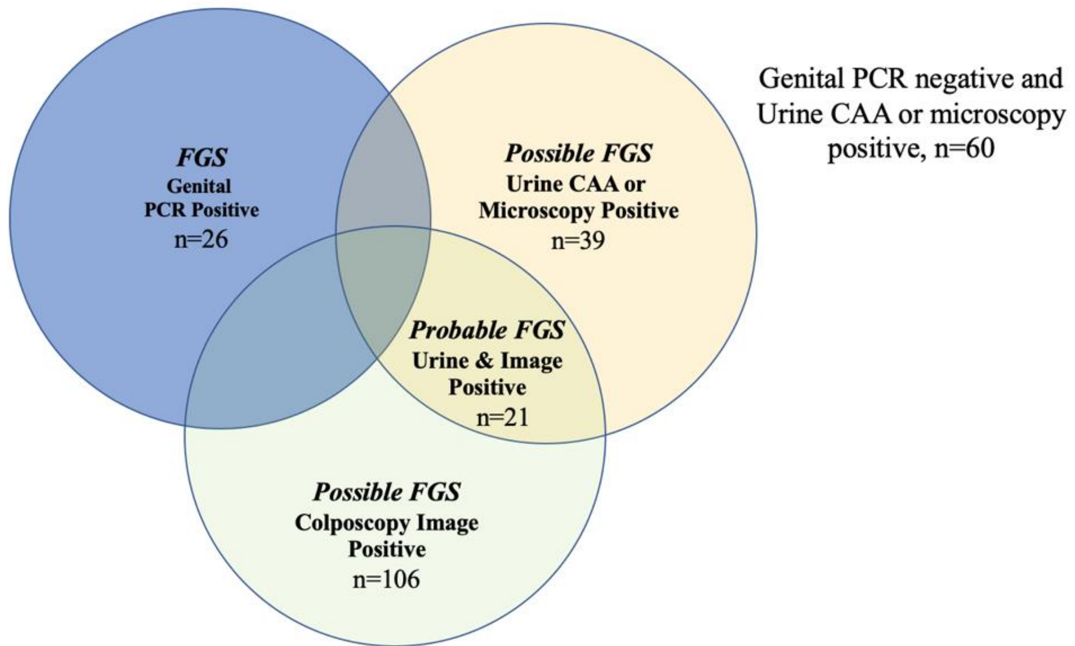
606

607 Figure 1

A



B



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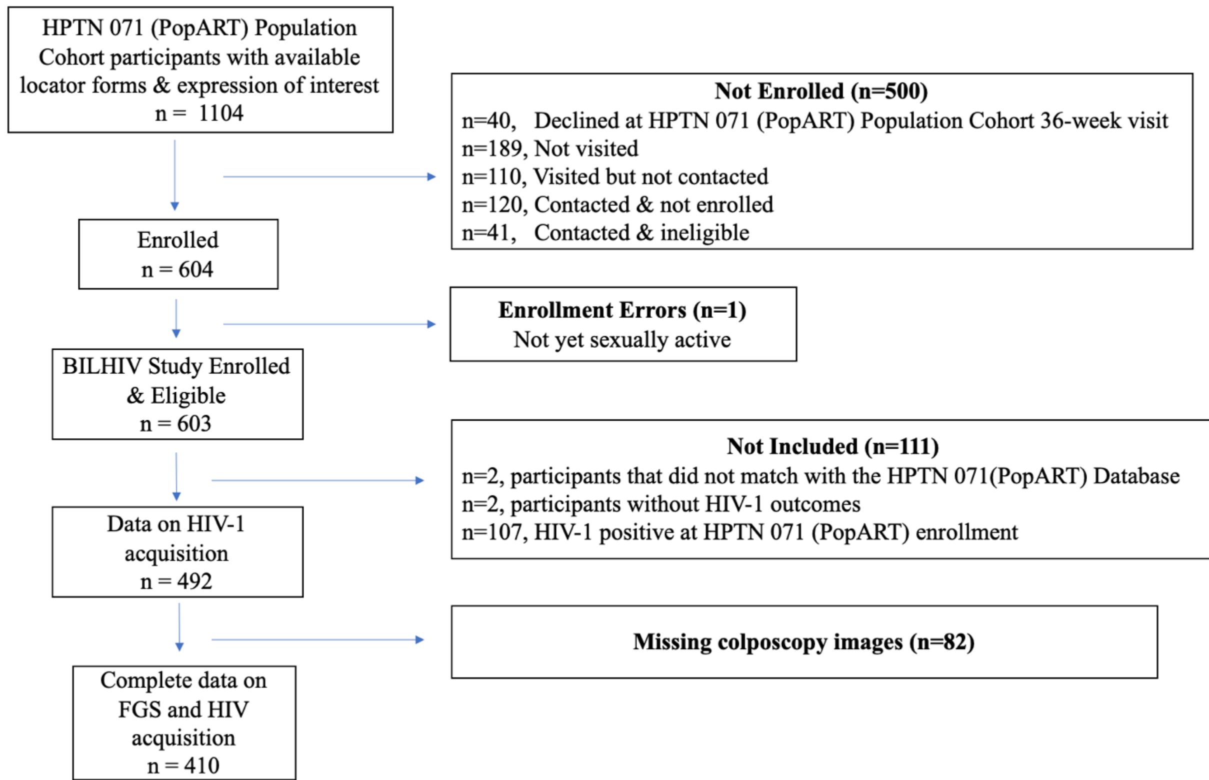
610 Figure 2

611



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