

## Schistosomiasis PCR in Vaginal Lavage as an Indicator of Genital *Schistosoma haematobium* Infection in Rural Zimbabwean Women

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**Abstract.** *Schistosoma* real-time polymerase chain reaction (PCR) is sensitive and specific in urine and stool. We sought to explore the relationship between genital schistosomiasis and the *Schistosoma* PCR in women. PCR was run on 83 vaginal lavage samples from a rural Zimbabwean population. Women underwent clinical and colposcopic investigations, analyses for sexually transmitted infections, and genital schistosomiasis. Thirty samples were positive for *Schistosoma* PCR: 12 were strong and 18 were weak positive. Sensitivity (67%) and specificity (83%) were best in women below the age of 25 years. A positive schistosome PCR result was associated with *S. haematobium* ova in genital tissue, so-called sandy patches, and bleeding. Prevalence determined by PCR were lower and real-time PCR values were weaker in older women. The presence of *Schistosoma* DNA may be greater in the recent lesions (e.g., in younger women). For diagnosis in rural areas and in large studies, *Schistosoma* PCR could become a supplement to gynecologic examinations.

### INTRODUCTION

Seven hundred million people are at risk of acquiring schistosomiasis, most of them in Africa.<sup>1</sup> Up to 75% of the women excreting *S. haematobium* eggs in urine have been found also to have schistosome eggs in the uterine cervix, vagina, or vulva.<sup>2,3</sup> Furthermore, women may have genital schistosomiasis without urinary findings. Genital schistosomiasis is associated with at least four genital mucosal manifestations, namely two types of sandy patches, abnormality in blood vessels, and contact bleeding.<sup>3–5</sup> The disease may cause discharge, genital itch, spot bleeding, and possibly also infertility and dyspareunia.<sup>3,6</sup> It has been suggested that schistosomiasis could be responsible for increased susceptibility to HIV, and a study in Zimbabwe reported a 3-fold higher odds ratio for HIV seropositivity in women with genital schistosomiasis, compared to those without.<sup>7–9</sup> This raises the possibility that treatment and prevention of genital schistosomiasis may have an impact on HIV transmission in areas where both infections are prevalent. Improvements in the detection of genital schistosomiasis would be an important component of such a strategy.

Currently female genital schistosomiasis is diagnosed by visual inspection of the cervicovaginal mucosa and on the microscopic detection of parasite eggs in biopsies, wet smears, or Papanicolaou (Pap) smears.<sup>4,10</sup> These investigations have limitations in resource-poor settings—they may be cumbersome and expensive, require rural electricity, water, and both clinical and laboratory equipment. The current gold standard for diagnosis is a biopsy.<sup>10</sup> However, the inflicted wound from a biopsy could pose a risk for HIV transmission for the patient or her partner and should therefore not be performed in routine investigations.<sup>4,11</sup>

Multiplex real-time polymerase chain reaction (PCR), including an internal control for the detection of inhibiting

factors, has been described for the detection of *Schistosoma mansoni* and *S. haematobium* DNA in stool or urine samples as an alternative for microscopic detection of parasite ova.<sup>12,13</sup> These PCRs have shown 100% specificity. Furthermore, the semi-quantitative outcomes of real-time PCRs defined by Cycle threshold (Ct)-values are significantly correlated with the microscopic egg counts in feces and urine. In subjects excreting more than 50 eggs per 10 mL of urine, a sensitivity of 100% has been described using the internal-transcribed-spacer-2 (ITS-2) based *Schistosoma*-genus-specific real-time PCR (*Schistosoma* PCR) in urine samples.<sup>12</sup> In this paper we report *Schistosoma* PCR in vaginal lavage material for the diagnosis of female genital schistosomiasis.

### MATERIALS AND METHODS

**Study subjects and area.** The study was conducted from October 1998 to March 1999 in Mupfure area in Mashonaland Central Province in Zimbabwe and has been described in detail previously.<sup>4</sup> Briefly, local village health workers listed female household members in the area assumed to be sexually active. All listed women aged 15–49 years residing in the indicated area were invited to take part in the study. Eighty-three percent attended from the area directly around the clinic and at three pick-up points, whereas there was a 33% attendance from the surrounding areas. Groups were comparable in waterbody contact, sexually transmitted diseases, and marital status. Virgins, pregnant, postmenopausal or menstruating women, those who refused to undergo gynecological examination or participate, and those who had other serious diseases were excluded.

**Ethical considerations.** Inclusion in the study took place after individual informed oral consent. In addition permission was given by the Provincial and District Medical Directors and by the village headman at village meetings. Ethical approval was given by the Medical Research Council of Zimbabwe (MRCZ/A/578) and by the ethical committee of the Special Program for Research and Training in Tropical Diseases Research (TDR, UNDP/WB/WHO) provided that biopsies were taken only upon an unconfirmed suspicion of

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malignancy because lesions may pose an entry port for HIV and many women in these areas may be in relationships where they are not able to refuse sexual intercourse.<sup>14</sup>

All included and excluded patients found positive for urinary or genital schistosomiasis were treated with praziquantel 40 mg/kg in one dose.<sup>15</sup> Patients with symptoms or signs of sexually transmitted infections (STI) or other diseases were treated in accordance with the Zimbabwean standard syndromic approach and referred to tertiary institutions when necessary.<sup>15</sup> Partner-treatment-slips were given to the index STI case; these ensured free treatment.

**Clinical examinations and sampling.** At the time of the examination, the clinician did not know the result of *S. haematobium* examination in urine. Patients were interviewed in Shona (the local language). Examination was commenced with cervicovaginal lavage collected by spraying saline (5 mL) on the vaginal wall and cervix twice, whereupon it was drawn back into a long syringe, from the posterior fornix, and deposited into four tubes and stored at -20 Degrees Centigrade. Subsequently photocolposcopic examination (Leisegang Photocolposcope, Script-O-Flash, Germany, Magnifications 7.5; 15; 30) was performed using autoclaved equipment. The mucosal and vulval surfaces were inspected section by section according to a predefined protocol. Homogenous yellow sandy patches were defined as sandy looking areas with no distinct grains using 15-times magnification. Grainy sandy patches constituted grains (~0.05 by 0.2 mm) situated in the mucosa. Neo-vascularization was defined as pathological convoluted (cork-screw), reticular, circular and/or branched, uneven-caliber blood vessels visible (by 15 times magnification) on the mucosal surface. Female genital schistosomiasis (FGS) is defined as the presence of sandy patches and/or ova in genital tissue. Contact bleeding was defined as fresh blood originating from the mucosal surface. Pre-contact bleeding was defined as darkened blood on the mucosal surface in the absence of recent or present menstruation. As reported previously, some of the clinical phenomena were also associated with other diseases (e.g., homogenous yellow sandy patches were also associated with herpes simplex virus type-2 (HSV-2) and neovascularization was associated with cervical intraepithelial neoplasia).<sup>4</sup> Pap smears were taken from all consenting women with a wooden spatula. Wet mounts were taken from consenting women who showed debris or friable, loose, and bleeding tissue with a titan autoclaved spatula.<sup>4</sup> Punch biopsies were taken only from consenting women when there was suspicion of malignancy. The investigation was finalized by bimanual examination.

Urine samples were collected on 3 consecutive days and 10 mL of each sample were processed by filtration for microscope examination for *Schistosoma* ova.<sup>16</sup> Single stool samples were collected from women and processed by Kato technique for the detection of *S. mansoni* ova.<sup>17</sup> Finally, a venous blood specimen was collected. Serum was separated by centrifugation and stored at -20 degrees centigrade immediately after collection.<sup>18</sup> Slides and tubes were not recycled.

**Analysis of genital samples.** When there was an adequate amount of remaining sample, laboratory analyses for STIs were performed. Women were tested for a median of seven STIs (range 1–8). Vaginal lavages samples were used for preparation of Gram-stained smears for detection of *Candida* and for bacterial vaginosis, with the latter defined using Nugent's criteria.<sup>19</sup> Pap smears were investigated for cell atypia and *S. haematobium* ova. Each biopsy was divided into two sections; one section was

sent for histology and the other was retained to make a crushed biopsy and examined microscopically for ova.<sup>4,10</sup> Wet smears were inspected by the field laboratory technician and by the clinician. Biopsies were explored by two independent pathologists and by the field clinician. Pap smears were inspected by an independent cytologist, by the field laboratory technician, and the clinician.

**Polymerase chain reaction on genital samples.** Vaginal lavage specimens were collected from 557 rural Zimbabwean women and 83 (15%) were available for *Schistosoma* real-time PCR analyses (Figure 1). The rest were excluded after a number of non-systematic, arbitrary events which have been described previously.<sup>18</sup> DNA was isolated from sediment of centrifuged vaginal lavage with the Wizard Genomic DNA Purification kit (Promega, Leiden, The Netherlands) according to the manufacturer's protocol. The presence of DNA was confirmed by  $\beta$ -globin PCR using primers PC03/04.<sup>20</sup> PCR was used to detect *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Haemophilus ducreyi*, and human papillomavirus.<sup>18</sup>

Ten years after the initial sampling in Zimbabwe, real-time PCR for the *Schistosoma* genus was performed on the remaining specimens.<sup>12,18</sup> Primers Ssp48F (5'-GGT CTA GAT GAC TTG ATY GAG ATG CT-3') and Ssp124R (5'-TCC CGA GCG YGT ATA ATG TCA TTA-3') were used to amplify a 77-bp fragment of the internal transcription spacer (ITS) 2 (homologous to both *S. haematobium* and *S. mansoni*), which was detected by the double labeled probe Ssp78T (FAM-5'-TGG GTT GTG CTC GAG TCG TGCC-3'-Black Hole Quencher) (Biolegio, Malden, The Netherlands). For detection of inhibitory effects during the amplification process, DNA of Phocine Herpes Virus 1 (PhHV) was added to the PCR mixture and detected with PhHV-1 specific primers and the specific double labeled probe (Biolegio).<sup>21</sup> Species specific differentiation between *S. haematobium* and *S. mansoni* were made with the PCR targeting the cytochrome c oxidase (*cox1*) gene.<sup>12</sup> Amplification, detection, and data analysis were performed with the Applied Biosystems 7500 (Applied

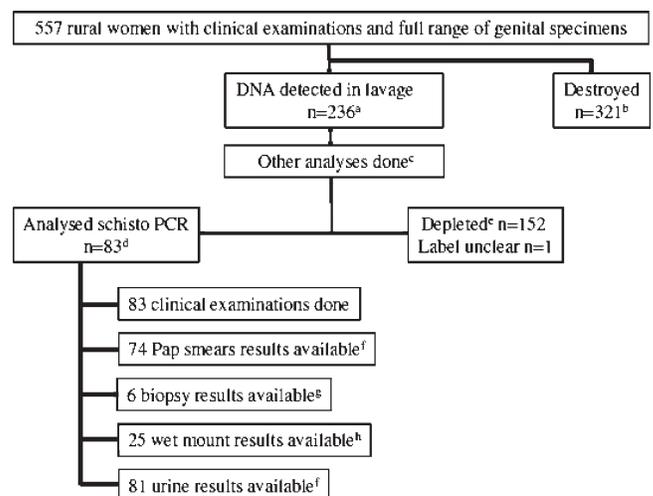


FIGURE 1. Recruitment of cases for *Schistosoma* real-time PCR analysis in rural Zimbabwean women. <sup>a</sup>Tested by  $\beta$ -globin PCR using primers PC03/04. <sup>b</sup>Breakdown of two freezers under electricity fluctuations in Zimbabwe. <sup>c</sup>Human papillomavirus PCR. <sup>d</sup>*Schistosoma* PCR detection.<sup>12</sup> <sup>e</sup>Not enough sample material left after previous analyses. <sup>f</sup>Done in all consenting women, some results were lost due to mistakes on/destruction of forms. <sup>g</sup>Done on suspicion of malignancy only. <sup>h</sup>Done in the presence of mucosal bleeding only.

Biosystems, Foster City, CA) Real-Time PCR system and Sequence Detection Software version 1.2.2.

**Serology.** Serology was used to detect antibodies against herpes simplex virus type 2 (HSV-2), *Trichomonas vaginalis*, and *Treponema pallidum* (Rapid plasma reagin and *Treponema pallidum* hemagglutination assay). Active syphilis was defined as the presence of an ulcer and seroconversion during follow-up and/or a positive IgM serological test. All sera were tested for HIV with two test kits; a third kit was used in the event of discrepant results (Recombigen, Cambridge Biotech, Galway, Ireland; Genelavia Mixt, Sanofi Diagnostics Pasteur S.A., Marnes La Coquette, France; Vironostica HIV, Organon Technika, Boxtel, The Netherlands).

**Statistical methods.** Chi-square, Fisher's exact tests (for numbers below 5), and odds ratio (OR) with 95% confidence interval were used when studying the association between laboratory results and clinical pathology in the genitals. To study simultaneously the impact of several variables, logistic regression analysis was applied on variables with a 20% significance level in bivariate analysis. The variables were excluded from multivariate analyses if they constituted less than 10 cases in denominator or numerator. Semi-quantitative *Schistosoma* real-time PCR results were categorized as strong (Ct  $\leq$  30), weak (Ct  $>$  30 and  $\leq$  50), or negative (undetected Ct-value redefined as 50). Sensitivity and specificity of the PCR were calculated using the combined results of the clinical and parasitological findings as gold standard. The diagnostic values of the available techniques were compared. The statistical analysis was computed using SPSS (Statistical Package for the Social Sciences, Chicago, IL) version 14.0 and EpiInfo2000 (Center for Disease Control, Atlanta, GA).

## RESULTS

Table 1 shows that PCR-tested and untested women had similar levels of urinary and genital schistosomiasis, current water contact, and HIV infection status. The mean age of the tested group was 2 years younger than the untested.

In 83 samples, 30 (36.1%) were positive for *Schistosoma* real-time PCR: 12 samples were strong positive Ct values (range 23.0–30.0; median 25.5) and 18 were weak positive (range 32.1–39.6; median 36.4). Table 2 shows that presence of *Schistosoma* DNA was significantly associated with sandy patches and *S. haematobium* ova in genital specimens. Of the 30 women with homogenous yellow sandy patches, 17 (56%) were found to have *Schistosoma* DNA, likewise *Schistosoma*

DNA was found in 10/19 (52%) of those with grainy sandy patches and 17/32 (53%) of those with neovascularization. Eleven of the 12 vaginal lavage positive with strong Ct values were found to have sandy patches. However *Schistosoma* DNA was not associated with polyps, ulcers and erosions, leukoplakia, and papillomatous tumors, together or separately.

When adjusted for HSV-2 and age, homogenous yellow sandy patches remained associated with *Schistosoma* DNA (adjusted odds ratio with 95% confidence interval, Adjusted OR 3.1, 95% CI (1.48–6.51),  $P = 0.003$ ). Likewise, neovascularization was associated with *Schistosoma* DNA after adjusting for cervical intraepithelial neoplasia, HSV-2, and age (Adjusted OR 3.1, 95% CI (1.5–6.4),  $P = 0.002$ ). After adjusting for high-risk human papillomavirus, HSV-2, and age, *Schistosoma* DNA was no longer significantly associated with contact bleeding (Adjusted OR 1.84, 95% CI (0.91–3.73),  $P = 0.091$ ). Grainy sandy patches were not associated with any other reproductive tract diseases, therefore no multivariate analysis was run for this clinical finding.<sup>4</sup> None of the other findings in Table 2 associated with the *Schistosoma* real-time PCR results were affected after adjusting for age (data not shown).

Seven cases were negative by *Schistosoma* real-time PCR even if *S. haematobium* ova were found in the genital specimens with microscopy (Table 2). Their ages were evenly distributed from 18–46 years of age and all had current waterbody contact. Five of these seven cases each had at least one of the sandy patch types and three of these also had urinary schistosomiasis. The two that did not have sandy patches were found to have ova by biopsy; both had childhood water contact, lived in the area, and had never been treated. One had urinary schistosomiasis and the other had neovascularization and contact bleeding. Sandy patches were found in 17 *Schistosoma* PCR negative cases. All but one was using the river currently, and the last had waterbody contact just 6 months prior, as well as during childhood. Using the combined results of the clinical and parasitological findings as a gold standard, *Schistosoma* genus real-time PCR had a 70% positive predictive value (PPV) for genital schistosomiasis, a sensitivity of 53% and a specificity of 79%. In the young women below the age of 25, the values were slightly better: PPV was 77%, sensitivity 67%, and specificity 83%. Younger women had stronger Ct values (data not shown). Figure 2 shows that the prevalences of *Schistosoma* genus PCR decrease with age even though the clinical findings were equally prevalent in all age groups.

*S. mansoni* ova were found in 14/83 (17%) stool samples, mean intensity four eggs/g (SD = 5). No *S. mansoni* was found

TABLE 1  
Comparing characteristics of women who were and were not tested by *Schistosoma* real-time PCR for genital schistosomiasis

	Schistosomiasis PCR* tested N	Not tested by PCR N	P
Urinary schistosomiasis† (%)	37/81 (46)	202/469 (43)	0.66
Urine mean egg count/10 mL (SD)‡	8.4 (28.9)	3.6 (16.2)	0.28
Current regular use of river (%)	76/83 (92)	413/473 (87)	0.25
Genital sandy patches§ (%)	37/83 (45)	219/474 (46)	0.78
HIV status (%)	20/83 (24)	139/470 (30)	0.30
Mean body mass index (SD)¶	21.9 (2.9)	22.2 (3.4)	0.74
Shona tribe   (%)	73/83 (88)	433/472 (92)	0.28
Married (%)	72/83 (87)	392/473 (83)	0.37
Mean age (SD)	31 (9)	33 (9)	0.034

\* *Schistosoma* genus PCR.<sup>12</sup>

† From all consenting women, some results were lost due to mistakes on forms, three consecutive urines.

‡ Only four cases had more than 50 ova/10 mL urine. SD = standard deviation.

§ The commonest current genital lesion in this population caused by *S. haematobium*.

¶ Calculated by dividing weight in kg by height in meters squared.

|| The main population group in the area, others were from Matabeleland, Mozambique, and Malawi.

TABLE 2  
*Schistosoma* PCR results' association with gynecological findings and urinary schistosomiasis in rural Zimbabwean women

	Schistosomiasis genus PCR*			P†
	High (N = 12)	Low (N = 18)	Negative (N = 53)	
All sandy patch types (below) (N = 37)	11	9	17	> 0.001
Homogenous yellow sandy patches‡ (N = 30)	10	7	13	> 0.001
Both grainy sandy patch types§ (N = 19)	5	5	9	0.057
Superficial grainy sandy patch§ (N = 9)	3	2	4	0.10
Deep grainy sandy patch¶ (N = 12)	3	3	6	0.216
Other findings				
Mucosal neovascularization   (N = 32)	8	9	15	0.007
Mucosal contact bleeding# (N = 24)	8	3	13	0.025
Mucosal pre-contact bleeding** (N = 14)	5	2	7	0.051
Mucosal oedema (N = 11)	5	1	5	0.017
Polypous tumor†† (N = 1)	0	0	1	0.49
Leukoplakia or papillomatous tumor‡‡ (N = 8)	0	2	6	0.30
Erosion or ulcer‡‡ (N = 11)	2	0	9	0.49
<i>S. haematobium</i> in any genital specimen (below)§§ (N = 17)	7	3/17	7/49	0.003
<i>S. haematobium</i> ova in Pap smear¶¶ (N = 6)	4	1/17	1/45	0.001
<i>S. haematobium</i> ova wet smear    (N = 9)	3/8	2/4	4/13	0.72
<i>S. haematobium</i> ova in biopsy## (N = 3)	1/1	0	2/5	0.84
<i>S. haematobium</i> ova in urine*** (N = 37)	9	8/17	20/52	0.029
HIV seropositivity (N = 20)	3	4	13	0.97

\* *Schistosoma* genus PCR detection, high: Ct (Cycle threshold) < 30, low: Ct 30–50, negative: Ct > 50.

† Test for trend (Mantel-Haenszel).

‡ Homogenous yellow sandy patches were sandy looking areas with no distinct grains using 15-times magnification.

§ Both deep and superficial oblong grains (~0.05 by 0.2 mm) situated in the mucosa.

¶ Covered with smooth mucosal membrane, seem to be deeper.

|| Convoluted (cork-screw), reticular, circular and/or branched, uneven-caliber blood vessels.

# Fresh, red intravaginal blood originating from mucosal surface after careful insertion of speculum.

\*\* Darkened intravaginal blood, not caused by recent menstruation.

†† Smooth pedunculated tumor originating from the cervix or vaginal walls.

‡‡ Also analysed separately (no difference found).

§§ *S. mansoni* was not found in genital specimens.

¶¶ Papanicolaou. Done in all consenting women, some results were lost due to mistakes on/destruction of forms.

||| Done in the presence of mucosal bleeding only.

## Done on suspicion of malignancy only.

\*\*\* Done in all consenting women, some results were lost due to mistakes on/destruction of forms.

in genital specimens. However *S. mansoni* DNA (*cox1*-based) was detected in one vaginal lavage sample, showing a weak Ct signal of 42. Likewise, *S. haematobium* DNA (*cox1*-based) was detected only in 18 of the 30 vaginal lavages with detectable *Schistosoma* genus DNA (ITS-2 based) (data not shown). The *cox1*-based *Schistosoma* PCRs had a lower sensitivity than the *Schistosoma* genus PCR (data not shown).

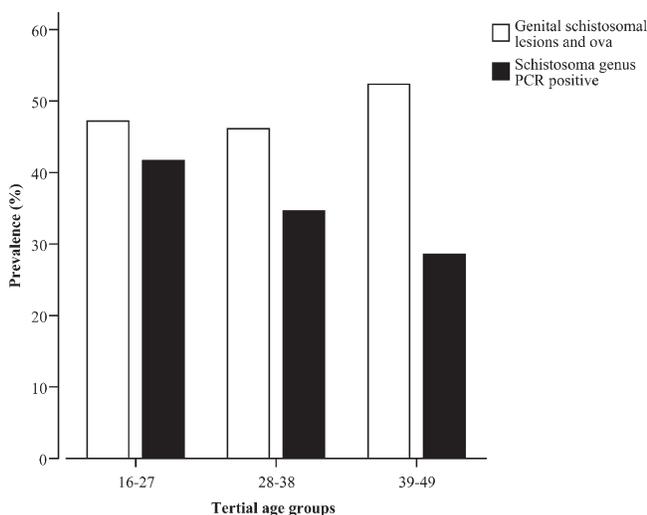


FIGURE 2. Age distribution of female genital schistosomiasis and *Schistosoma* genus PCR positive cases. Female genital schistosomiasis was equally present in all age groups whereas *Schistosoma* PCR decreases with age.<sup>4</sup>

## DISCUSSION

The presence of *Schistosoma* DNA is highly associated with the genital mucosal sandy patch types, neovascularization, and contact bleeding, also after correction for age and other diseases. Contrary to previous publications, but as has been indicated in this population previously, ulcers, polyps, papillomatous tumors, and leukoplakia were not associated with positive results from the *Schistosoma* real-time PCR assay.<sup>3,4</sup> These data indicate that the presence of genital *Schistosoma* DNA decreases with age in adult women, contrary to previous findings where genital schistosomiasis prevalence is the same in the different age groups.<sup>3,4</sup>

The results of the sensitivity and specificity of *Schistosoma* real-time PCR in vaginal lavage may shed some light on the pathogenesis of FGS and the sampling methods. Although better in detecting genital schistosomiasis than the other (ethically acceptable) laboratory tests, the *Schistosoma* PCR sensitivity was lower than 60%. False negative *Schistosoma* real-time PCR in lavage may be attributed to the non-invasive nature of the lavage compared with the other genital tests. Pap smears, wet smears, and biopsies harvest ova from the epithelium and deeper in the stroma and are, in order of appearance, more invasive than lavage.<sup>4,10</sup> The vaginal lavage itself was not examined microscopically for *S. haematobium* ova, consequently the lavage analysis is fundamentally different from the parasitological tests in feces and urine. Moreover the histopathological studies have shown that ova may be situated deeply in genital tissue, in stroma, rather than in epithelium, as was the case in this population as well.<sup>4,10</sup> *Schistosoma* DNA is

not necessarily found in lavage in persons with deeply situated ova. Furthermore, we cannot preclude that local bleeding or foreign material, such as intravaginal water, soaps, herbs, and sperm, may dilute and disturb the *Schistosoma* PCR test giving false negative results.<sup>4</sup> False positive cases may be caused by the fact that *S. haematobium* ova can also be found in sperm and history of sexual intercourse was not recorded in this study.<sup>22</sup> The contamination from the outer genitals by urine or contamination from previous analyses (e.g., the practice of recycling/reusing slides/filters) was not an issue in this study.

The infrequent case reports of genital *S. mansoni* infections, compared with those from *S. haematobium*, has been taken as an indication that *S. mansoni* probably rarely causes genital lesions in women.<sup>23,24</sup> In this rural Zimbabwean population, 17% were found to have *S. mansoni* in feces, however no *S. mansoni* ova were found in the genital specimens.<sup>4</sup> We cannot, however, preclude that some of the positive PCRs represent *S. mansoni*.

Previous publications on *Schistosoma* PCR have shown higher sensitivities and specificities for microscopically detected ova in stool and urine.<sup>12,13</sup> The urine report is from school children, and the stool report is from young adults (median age 20 years), whereas the median age was 33 years in this population. The current findings indicate that lesions, even superficial grains and inflammation, may be there in the absence of *Schistosoma* DNA. However, *Schistosoma* prevalences were lower, Ct values weaker in older women, and the positive predictive value of the test was better in the young. This may indicate that there may be more DNA in the younger age groups, irrespective of lesion size. Furthermore, the pathogenesis of the disease is not fully understood.<sup>25,26</sup> Viable ova may be found for a few weeks after parasite death but it is unknown for how long schistosome DNA persists. Previous reports suggest that genital schistosomal lesions are refractory to treatment (whereas urine ova excretion ceased). This may indicate that lesions do not heal even if ova have died or disintegrated. Similarly, several histopathological reports indicate that there may be inflammatory reaction also around dead ova, presumably PCR negative, yet continuing to pose a clinical problem.<sup>27,28</sup>

It has been suggested that young women should be treated to prevent adult lesions and HIV transmission.<sup>26</sup> The timing and frequency of such treatment has not yet been explored. Treatment of other reproductive tract diseases may decrease genital HIV-RNA shedding in co-infected cases, but this has not been explored for genital schistosomiasis.<sup>29-33</sup> Repeated anti-schistosomal mass treatment has been done in several countries and the effects are well-documented in the urinary tract.<sup>34</sup> However, there is a paucity of documentation on the genital tract, although one report indicates that there is a partial prevention of genital tract morbidity in those that were treated as teenagers.<sup>26</sup>

Genital schistosomiasis lesions are often not visible to the naked eye and the investigations are subject to inter- and intra-observer variation and the quality of the equipment<sup>4</sup> (personal communication, Dr. Bodo Randrianasolo, Madagascar). Adequate gynecologic investigations can be near to impossible in many rural areas. Many women are not accustomed to gynecological investigations, the clinic rooms may be inadequately designed for privacy or lighted conditions, the waiting queues may be prohibitive for thorough inspections, electricity supply may be erratic, and the turning of the speculum for inspection of the entire mucosa may be uncomfortable.<sup>4,35</sup>

For the pending research projects the *Schistosoma* real-time PCR could become a supplement to or even replace gynecologic

examinations. PCR machines are now available in many university hospitals in endemic areas, rural and mass investigations could hence be limited to intravaginal swabs if funds were available.<sup>4</sup> Furthermore, self-sampling for the diagnosis of human papillomavirus PCR has already been tested in several settings.<sup>36-38</sup> Although it is currently unrealistic to use *Schistosoma* PCR of vaginal lavage in ordinary clinical work in endemic areas this is a method that could be put to use in evaluation of treatment and research.

These new *Schistosoma* real-time PCR analyses seem to corroborate that lesions may become chronic after deposition and death of ova. Furthermore, the presence of *Schistosoma* DNA in the young indicates that this method could be used to follow them for effect of treatment. Further refining of the sampling methods and the use of intravaginal substances as a confounder should be explored. Female genital schistosomiasis as a cause of disease, infertility, and risk for HIV could be explored along with the effect of treatment. Likewise, the issues of *S. mansoni* and male genital schistosomiasis as risk factors for HIV transmission to women are pending. The *Schistosoma* real-time PCR is a good candidate for diagnosis in research and evaluation projects, maybe especially in the young.

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