

## 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

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**Abstract.** *Schistosoma haematobium* eggs and *Schistosoma* DNA levels were measured in urine samples from 708 girls recruited from 18 randomly sampled primary schools in South Africa. Microscopic analysis of two 10-mL urine subsamples collected on three consecutive days confirmed high day-to-day variation; 103 (14.5%) girls had positive results at all six examinations, and at least one positive sample was seen in 225 (31.8%) girls. *Schistosoma*-specific DNA, which was measured in a 200- $\mu$ L urine subsample by using real-time polymerase chain reaction, was detected in 180 (25.4%) cases, and levels of DNA corresponded significantly with average urine egg excretion. In concordance with microscopic results, polymerase chain reaction results were significantly associated with history of gynecologic symptoms and confirmed highly focal distribution of urogenital schistosomiasis. Parasite-specific DNA detection has a sensitivity comparable to single urine microscopy and could be used as a standardized high-throughput procedure to assess distribution of urogenital schistosomiasis in relatively large study populations by using small sample volumes.

### INTRODUCTION

Schistosomiasis is endemic to 76 countries and 85% of those infected live in rural areas of sub-Saharan Africa, where at least 100 million women and girls are at risk of having this helminth infection.<sup>1</sup> Those who are most vulnerable to infection are pre-school and primary school children, adolescent girls, and women of childbearing age. To reduce morbidity the World Health Organization recommends annual treatment of school-age children in areas of high endemicity.<sup>2</sup>

In sub-Saharan Africa, *Schistosoma haematobium* is the predominant species.<sup>3</sup> Adult worms lodge in the venules surrounding the pelvic organs, where they deposit their eggs. Some of the eggs are released into the urine whereas others get trapped in the bladder mucosa where they give rise to granulomatous inflammation causing hematuria and urinary symptoms. Up to 75% of the women who excrete *S. haematobium* eggs in the urine may also have *Schistosoma* eggs in the uterus, cervix, vagina, or vulva.<sup>4</sup> The genital manifestations may mimic cancer-like lesions and the different sexually transmitted diseases, such as ulcers, genital warts, polyps, and also cause mucosal immune activation and blood vessel friability.<sup>5–7</sup> Several studies have indicated that genital manifestation of schistosomiasis may make women susceptible to human immunodeficiency virus (HIV) infection and they may possibly also develop infertility.<sup>8–10</sup> Because *S. haematobium* affects the urinary and genital tracts, urinary schistosomiasis has been renamed urogenital schistosomiasis.<sup>11</sup>

The gold standard for diagnosing gynecologic schistosomiasis has been the demonstration of eggs in a crushed biopsy specimen.<sup>6</sup> However, this procedure is controversial because it is invasive and could make the cervical mucosa more susceptible to infections with other sexually transmitted infections,

such as those with HIV or human papillomavirus.<sup>4</sup> The colposcope for investigating gynecologic morbidity has limitations because it is highly observer-dependent, requires extensive training and expensive equipment, and cannot be used among children because intra-vaginal inspections are normally not performed before the onset of sexual activity.

For urinary tract infection with *S. haematobium*, microscopic examination for eggs is considered the diagnostic gold standard.<sup>12</sup> However, the extent to which the presence and intensity of excreted eggs in urine is associated with the actual degree of genital morbidity is still under debate because studies have shown a substantial proportion of adult women with genital lesions to be urine microscopy negative.<sup>4</sup> In adults, gynecologic symptoms are often seen many years after reported water exposure.<sup>4</sup> Conversely, in school-age girls, one would expect infections to be more recently acquired, with adult worms actively producing eggs that are deposited at different tissues in the pelvic region and excreted into the urine in relatively large quantities.

The association between urine microscopy-detected *S. haematobium* infection and genital symptoms has been recently studied on a group of 10–12-year-old school girls in South Africa.<sup>13</sup> One third of the interviewed girls reported to have a history of genital symptoms, and multivariate regression analysis showed a significant association with the urine microscopy results. To avoid suboptimal diagnosis and not to miss light infections, intense microscopy was performed as generally recommended, which required repeated urine sample collection and examination.<sup>12,13</sup> Microscopy has the limitations of being an observer-dependent procedure, as well as laborious, when applied to large-scale population based surveys. For this purpose there is a clear need for more standardized and highly sensitive high-throughput diagnostic procedures.<sup>14,15</sup>

In recent years, several nucleic acid-based diagnostic tests have been established for specific and sensitive detection and quantification of a broad range of parasite DNA in clinical

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samples, including an internal transcribed spacer (ITS)-based *Schistosoma*-specific multiplex real-time polymerase chain reaction (PCR).<sup>16,17</sup> In this study, we further explored real-time PCR as a diagnostic tool for mapping school-based distribution of urogenital schistosomiasis by comparing urine *Schistosoma* DNA levels in the same cohort of school girls from South Africa with the findings of multi-sampling microscopy, as well as reported gynecologic symptoms.

## MATERIALS AND METHODS

**Study population.** The study design is a school-based, cluster, randomized, cross-sectional study of girls 10–12 years of age in rural primary schools in KwaZulu-Natal, South Africa. Participants were recruited from 18 randomly selected schools, all situated in a coastal area of 5,866 km<sup>2</sup> within Ugu district, south of Durban.<sup>13</sup> The region is known to be endemic for *S. haematobium* and HIV.<sup>18,19</sup> Other common helminths are *Ascaris lumbricoides* and *Trichuris trichiura* but only occasional cases of infection with *S. mansoni* have been reported.<sup>20</sup> Recruitment of the study participants and their clinical symptoms have been described in detail elsewhere.<sup>13</sup> In brief, data and sample collection were performed during September 2009–November 2010. Before the study, information meetings were organized at each school and girls were invited to participate if their parents provided consent.

**Interview.** Research assistants invited consenting girls to face-to-face private interviews performed in the local language isiZulu. In brief, in this study we used four key variables: 1) living with both parents, 2) reported water contact, 3) history of red urine, and 4) history of genital symptoms because these variables potentially reflect social status, exposure to and clinical outcome of *S. haematobium* infection.

Each girl was questioned about her living conditions and relationship with her biological parents and about her own observations concerning red urine. High-risk water contact was defined as reported regular exposure to potentially infective water bodies covering at least 10% of the body surface or being in the risk water at least 60 minutes per exposure. History of genital symptoms consisted of reported symptoms of bloody discharge, malodorous discharge, genital itch or burning sensation in the genitals, and some additional symptoms, which were only occasionally mentioned. Further details about the interview are given elsewhere.<sup>13</sup>

**Ethical considerations and permissions.** This study project drew from a larger project that was approved by the Biomedical Research Ethics Administration, University of KwaZulu Natal (Ref BF029/07). The Departments of Health and Education (Ref HRKM010-08) of KwaZulu Natal also approved the study. Ethical permission was also granted from The Norwegian Ethics Committee, Regional Etisk Komité Øst-Norge (REK-Øst), (Ref IRB 0000 1870) and The European Group on Ethics in Science and New Technologies (Ref IRSES-2010:269245).

Parents or guardians provided written informed consent and consent was given by each girl. All girls were informed of the right to withdraw and to abstain from answering questions without negative consequences. To protect children from stigmatization, the disease was discussed in general terms as urinary schistosomiasis, known as isichenene in isiZulu. Treatment of schistosomiasis with praziquantel was offered to all participants, and all were informed about possible side

effects. Support of a private psychologist was available to assist with cases that required psychological support. The project also had a referral system with local clinics and hospitals for those participants that required this system. Consent forms were returned and signed by 92% of the parents in the first 13 schools, but because of teacher strikes, the turnout was only 17% in the remaining five schools.<sup>13</sup>

**Sample collection procedure and microscopy.** A team of trained field research assistants and school nurses visited each school for general study information and urine collection between 10:00 AM and 2:00 PM on three consecutive days. Samples were transported to the laboratory on the same day in dark cooler boxes to ensure optimal processing. After arrival, two 10-mL urine samples (A and B) were preserved with 1 mL of 2% tincture of merthiolate in 5% formalin solution.<sup>21</sup> Within the same week, samples were centrifuged for 10 minutes at 4,000 rpm and microscopically investigated at a magnification of 10× for *S. haematobium* eggs. The egg counts were recorded for each 10 mL of urine separately. When more than 1,000 eggs were seen, counting was stopped. Microscopy was performed blinded to previous results and by separate technicians. Quality control was performed by an independent senior microscopy expert on 10% of randomly chosen samples.

**Detection of *Schistosoma* DNA.** Aliquots of 1 mL of each first-day urine sample, before the merthiolate-formalin was added, were transferred into cryotubes. The aliquots were stored at 4°C for maximum of one week in the dark and thereafter stored at –80°C for several months, before being transported to the Netherlands in frozen conditions for DNA isolation and detection. For a subselection of 85 urine samples, 49 of them that were microscopy negative at all examinations, a second aliquot was tested by PCR at a local laboratory in South Africa.

In the Netherlands, DNA isolation and the set-up of the PCR was performed at the Leiden University Medical Center with a custom-made automated liquid handling station (Hamilton, Bonaduz, Switzerland). DNA was isolated from a 200-μL subsample of each urine sample by using a proteinase K heating step and QI Amp spin columns (QIAGEN, Hilden, Germany) as described.<sup>16</sup> Phocin herpes virus 1 (PhHV-1) was added to the lysis buffer as an internal control.<sup>22</sup> For real-time PCR, *Schistosoma*-specific primers Ssp48F and Ssp124R were used to amplify a 77-basepair fragment of ITS2. The double-labeled probe Ssp78T was used to detect amplification.<sup>16</sup> For the internal control, PhHV-1-specific primers PhHV-267S and PhHV-337AS and the specific double-labeled probe PhHV-1-305TQ were included in each reaction mixture. The amplification and detection of each DNA sample, with use of the Bio-Rad (Hercules, CA) CFX Manager, was performed as described.<sup>16</sup>

Similar DNA isolation and PCR procedures were used at the laboratory in South Africa, with some minor modifications. All steps were done manually. The PhHV-1 internal DNA control was replaced by Lambda DNA (1 μL Lambda DNA; dilution of 10<sup>-6</sup> per 400 μL of lysis buffer). Therefore Lambda-specific primers and the Lambda-specific CAL Fluor Orange 560-labeled probe Lambda-TM<sub>p</sub> were used in the reaction mixture. The amplification of each DNA sample was performed in a 25-μL reaction mixture containing PCR buffer (containing 10× Gold Buffer, dNTPs, and TaqGold), 5 mM MgCl<sub>2</sub>, 2.5 μg bovine serum albumin (Roche Diagnostics

Nederland BV, Almere, The Netherlands), 1.5 pmol of each *Schistosoma*-specific primer, 1.5 pmol of each Lambda-specific primer, 1.25 pmol of each of the *Schistosoma*-specific and Lambda-specific probes, and 5 µL of the DNA sample. The thermocycler was set for 15 minutes at 95°C, followed by 50 cycles, each for 15 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C. Amplification, detection, and data analysis were performed with the Corbett Rotor Gene 3000 (QIAGEN).

In both laboratory settings, the PCR output consisted of a cycle threshold ( $C_t$ ) value, which represented the amplification cycle in which the level of fluorescent signal exceeded the background fluorescence and thereby indicating the presence of parasite-specific DNA in the sample that was tested. The  $C_t$  values of the internal controls (PhHV in the Netherlands, Lambda DNA in South Africa) were within the expected range for all samples and will not be further discussed within the results.

**Data analysis and statistical testing.** The PCR analysis was performed blinded from microscopy or other field data. The results of the real-time PCR analysis were stored and grouped in a Microsoft (Redmond, WA) Access database and imported into IBM (Chicago, IL) SPSS 20.0 for statistical analysis together with provided field data, including results of the interviews and urine microscopy examinations.

Based on microscopy, the infection was classified as high intensity if the mean number of eggs of the three specimens was > 50 per 10 mL of urine.<sup>23</sup> Based on PCR, the infection was classified as high intensity ( $C_t < 30$ ), medium intensity ( $30 \leq C_t < 35$ ) or low intensity ( $35 \leq C_t < 50$ ).<sup>15</sup> These categories were chosen arbitrarily, based on previous experiences with protozoal and helminth infections where DNA loads with a  $C_t$  value < 30 could generally be microscopy confirmed and DNA loads with a  $C_t$  value > than 35 were always microscopy negative. For better visualization in the scatter plots,  $C_t$  values were also recalculated into arbitrary units (AU) of copy numbers of *Schistosoma* DNA. An arbitrary value of 1 AU was assigned to each positive sample with a  $C_t$  value  $\geq 35$ . Assuming 100% efficacy of the DNA multiplication process up to 35 cycles, a duplication of AU was calculated for each PCR cycle, meaning for each single  $C_t$  value reduction starting with a  $C_t$  value of 35.

Because the data were not normally distributed, results were described as the total number, range, interquartile ranges (IQRs), and median value of positive subjects. For the evaluation of *S. haematobium* infection intensity, concordance between sample egg counts and PCR output (AU) was statistically analyzed by using the non-parametric Spearman's rank order correlation coefficient ( $\rho$ ) because both variables were skewed even after log transformation. The McNemar statistical test was used to analyze concordance between the different diagnostic procedures. Statistical significance was considered at  $P < 0.05$ .

## RESULTS

**General characteristics of the study population.** A summary of the sample selection procedure is shown in Figure 1, and a general description of the study population is shown in Table 1. A total of 1,948 school girls were included for participation. However, some participants were excluded because unreturned consent forms, refusal to participate, absenteeism,

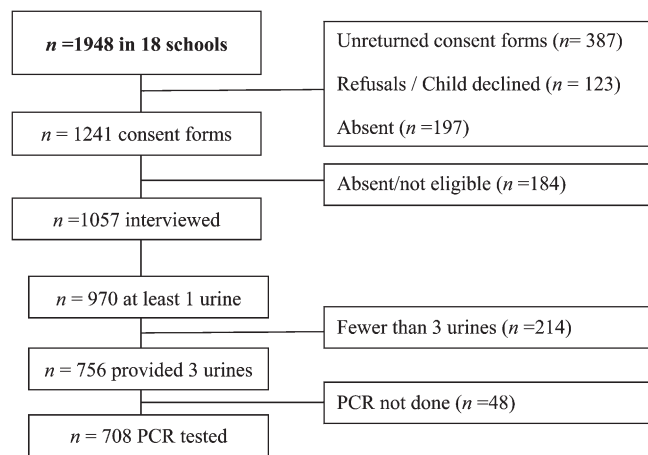


FIGURE 1. Flow chart of study participation and adherence by 708 school girls in KwaZulu Natal, South Africa. PCR = polymerase chain reaction.

and not being within the specified age range. Of the interviewed girls, 708 pupils provided urine samples for complete microscopy data and PCR, and 13 schools had more than 20 participants. Discrepancy analysis showed no relevant differences between the 708 girls tested and those excluded because of incomplete sampling. At the interview, 27.5% of the 708 girls reported not living with their parents, 60.4%

TABLE 1  
Epidemiological and parasitological characteristics of urine *Schistosoma* PCR tested school girls (n=708)

Characteristics	n	(%)	Range
Number	708		
Age (years)			
Median (range)	11		10–12
Number of schools	18		
Participants per school			
Median (range)	33		4–158
<b>Reported during interview<sup>1)</sup></b>			
Situation at home <sup>2)</sup> :			
Living with both biological parents	163	(23.1)	
Living with one biological parent	348	(49.4)	
Possible exposure to infected water:			
Low risk water contact	180	(25.4)	
High risk water contact	248	(35.0)	
Red urine:			
Sometime before	50	(7.1)	
Last week	60	(8.5)	
Genital symptoms <sup>3)</sup> :			
History of bloody discharge	52	(7.2)	
History of burning sensation in the genitals	101	(14.3)	
History of any symptoms	231	(33.1)	
<b>Urine microscopy (<i>S. haematobium</i> eggs)</b>			
60 mL examination	225	(31.8)	
Positive in all 6x 10 mL examinations	103	(14.5)	
Showing mean egg count > 50 eggs/10mL	60	(8.5)	
10 mL examination (day 1)			
<i>S. haematobium</i> egg positive cases detected	171	(24.2)	

1) Full details are presented in Hegertun *et al.* (2013) definitions describes at material and methods section.

2) In 3 cases no answer was given.

3) In 5 cases no answer was given.

reported possible exposure to infested water, 15.6% reported a history of red urine, and 33.1% reported any history of genital symptoms (Table 1). These characteristics were within the same range as the answers given by all 1,057 interviewed girls, being 27.3%, 62.6%, 17.8% and 35.0%, respectively.

**Schistosoma microscopy.** The day-to-day variation in counted *S. haematobium* eggs is shown in Figure 2A, which depicts egg counts of sample day 1A and sample day 3A. Intensity of infection between the two sample readings correlated significantly in those positive ( $n = 195$ ; Spearman's  $\rho = 0.32$ ,  $P < 0.001$ ). At the same time, discrepancies were seen. Although 139 girls were microscopy positive for both samples, 32 girls were positive in urine sample 1A and negative in sample 3A. In 24 girls, the opposite result was seen. Less than 50 eggs/10 mL of urine were counted in 43 of the 56 girls; there was only one

positive urine sample. Identical patterns were seen when microscopy data for other urine readings were compared.

The overall prevalence of *S. haematobium* based on the detection of eggs in urine samples collected on three consecutive days was 31.8%, with an average egg count of 0.17–624 eggs/10 mL (median = 20, IQR = 5–55). One hundred and three (45.8%) of the 225 microscopy-positive girls showed eggs in all six urine examinations, and average high intensity infections were seen in 60 (26.7%) infected girls. Based on readings from the three consecutive days, the prevalence of each day was 25.8–26.4%, and based on the individual 10-mL urine examinations, the prevalence was 22.5–24.2%.

**Schistosoma PCR.** The PCR analysis of first day urine samples showed *Schistosoma* DNA in 25.4% of the samples,

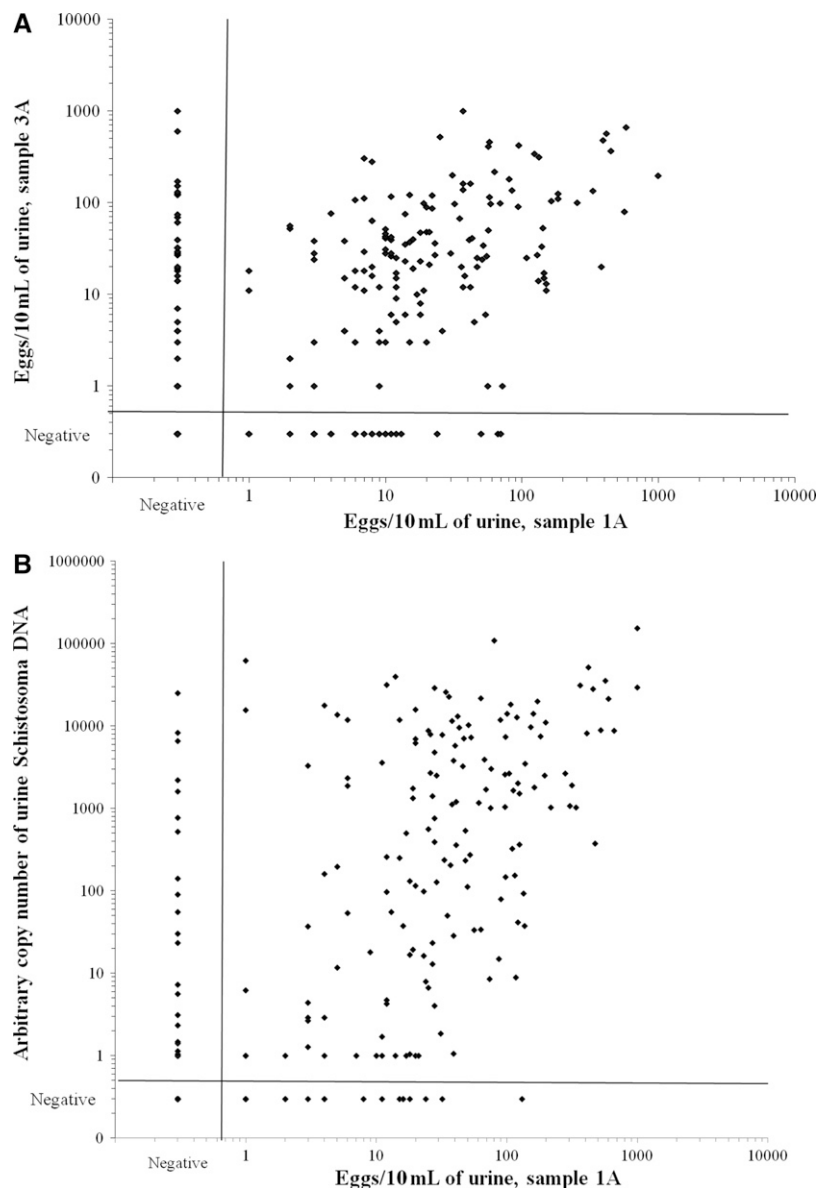


FIGURE 2. Scatter plots of 708 school girls 10–12 years of age in KwaZulu-Natal, South Africa. (A) Comparison of urine microscopy of the first 10-mL sample of day 1 (sample 1A) and the first 10-mL sample of day 3 (sample 3A). (B) Comparison of urine microscopy of the first 10-mL sample of day 1 (sample 1A) and urine *Schistosoma* DNA loads determined in the same day sample (200  $\mu$ L) by using real-time polymerase chain reaction (PCR).



and 125 of these 180 samples had high DNA loads. The AU of DNA copies ranged from 1 to  $154 \times 10^3$  (median = 448, IQR =  $12-7 \times 10^3$ ). The association between urine *Schistosoma* DNA load quantified in 200- $\mu$ L samples and the number of eggs counted in a 10-mL sample of the same portion is shown in Figure 2B. A significant correlation was seen between the two procedures ( $n = 199$ ; Spearman's  $\rho = 0.51$ ,  $P < 0.001$ ). Discrepancies were also observed. Nineteen samples were PCR negative and microscopy positive, all samples except one had egg counts  $< 50$  eggs/10 mL. In addition, *Schistosoma* DNA was detected in 28 urine samples that were microscopy negative. In 14 of these samples, eggs were seen in at least one of the five other 10-mL urine samples by microscopy, and the remaining 14 had low DNA levels, which indicated that half of them had 1 AU and the maximum seen was 90 AU. The number of detected cases by urine PCR did not statistically differ from the number of detected cases by each of the 10-mL urine microscopy examinations, or each of the three consecutive day readings, but was significantly less compared with the 225 cases detected when all microscopy readings were combined. Also, the average egg counts correlated significantly with the measured urine AU of DNA copies ( $n = 239$ ; Spearman's  $\rho = 0.70$ ,  $P < 0.001$ ).

A comparison between PCR outputs in the two PCR laboratories showed only minor discrepancies. Four of the 36 microscopy-positive urine samples were missed in South Africa; these samples showed positive results in the Netherlands. Conversely, 1 of the 49 microscopy-negative samples had a high DNA level in South Africa, which could not be confirmed when tested in the Netherlands.

**School level analysis.** To fully explore the diagnostic value of urine PCR to identify high-risk communities, findings were further analyzed at school level. Data for 13 schools with at least 20 participants per school, representing 666 school girls, were aggregated. The prevalence of *S. haematobium* per school based on urine PCR ranged from 3.4% to 45.9% (median = 27.0%, IQR = 12.0–37.8%) and showed a significant correlation with the prevalence range based on complete microscopy ( $n = 13$ ; Spearman's  $\rho = 0.95$ ,  $P < 0.001$ ). The prevalence of detectable *Schistosoma* DNA showed a significant correlation with reported history of genital symptoms at school level (Figure 3) ( $n = 13$ ; Spearman's  $\rho = 0.74$ ,  $P < 0.01$ ). Also, reported history of high-risk water contact and history of red urine showed a significant correlation with school-based *Schistosoma* prevalence determined by PCR or microscopy ( $n = 13$ ; Spearman's  $\rho = 0.62-0.73$ ,  $P < 0.03$ ). A total of 58.6–83.8% of the girls reported living with at least one parent, and 13.9–34.5% reported living with both parents. No association was seen between household composition and prevalence of schistosomiasis determined by PCR or microscopy.

## DISCUSSION

In this study, we used a PCR with 708 urine samples collected from girls in 18 primary schools from KwaZulu-Natal, South Africa. Microscopy was performed six times on a 10-mL urine sample collected over three consecutive days. We found substantial dispersion of counted eggs in urine;  $< 15\%$  of the girls showed positive results for all six 10-mL urine sample examinations. This high day-to-day variability in *Schistosoma*

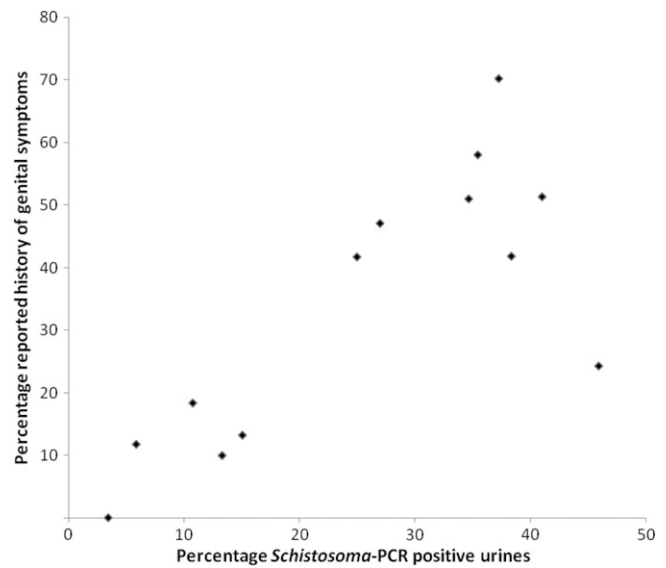


FIGURE 3. Scatter plot comparing school-based prevalence of *Schistosoma* DNA in urine samples and reported history of genital symptoms in school girls in KwaZulu Natal, South Africa. Schools ( $n = 13$ ) with at least 20 participants were included, which included 666 school girls of 10–12 years of age. PCR = polymerase chain reaction.

eggs shedding is generally acknowledged, but data have been published only sparsely.<sup>24</sup>

Interestingly, we found the sensitivity of the ITS-based PCR performed with a 200- $\mu$ L subsample to be comparable to that of each of single 10-mL microscopy examinations. However, the sensitivity was lower when compared with repeated microscopy (complete 60-mL urine sample examination). DNA amplification was detected in 98.3% and 88.3% of the samples with  $> 50$  eggs/10 mL of urine and  $\leq 50$  eggs/10 mL of urine, respectively, and in 28 (5.2%) of 537 urine samples in which eggs were not detected at the first 10-mL urine sample examination. In one considers that our population of school girls was truly infected if at least one of the six microscopy examination results was positive, or if *Schistosoma* DNA was detected in urine, this resulted in a negative predictive values for complete 60-mL microscopy, 10-mL microscopy, and urine PCR of 98%, 86%, and 88%, respectively.

In a previous study, the same ITS-based real-time PCR for specific detection of *Schistosoma* DNA showed excellent sensitivities for 730 urine samples collected from children in five primary schools from different communities in the Greater Accra region of Ghana.<sup>17</sup> Compared with our study, infection levels were low in the selected schools in Ghana; the overall prevalence was 7.8% and only 10 of 57 children had  $> 50$  eggs/10 mL of urine. *Schistosoma* DNA was amplified in 100% and 85.2% of urine samples with  $> 50$  eggs/10 mL of urine and  $\leq 50$  eggs/10 mL of urine, respectively. In addition, *Schistosoma* DNA was detected by PCR in 102 (15.2%) of 673 urine samples in which eggs were not detected by single 10-mL urine microscopy. These findings resulted in higher negative predictive values for real-time PCR ( $> 94.6\%$ ) than for microscopy (54.3–95.7%).<sup>17</sup>

Although *Schistosoma* DNA was quantified in both studies according to the same PCR procedure, microscopy showed better performance in South Africa. Results could not be explained by the higher overall *S. haematobium* prevalence

in samples in South Africa because concordance between PCR output and microscopy did not shift between the selected schools (e.g., the five schools with a microscopy prevalence < 20% showed a similar range in prevalence based on urine *Schistosoma* DNA detection, while the five schools tested in Ghana showed substantial higher infection levels based on PCR).<sup>17</sup>

A possible explanation for the comparatively poor PCR performance in the current study could be the procedure of sample collection (i.e., the time lag between receiving the samples and definitive storage in a frozen condition). Because of logistics, freezing of samples could only be performed a number of days, up to a week, after collection. When mixed with ethanol, fecal samples can be kept at room temperatures for weeks to months, even in tropical conditions, before transported to centralized laboratory facilities for DNA isolation.<sup>25</sup> For urine samples, optimal storage conditions without immediate need for a cold chain still need to be further explored.

Alternatively, the ITS-based PCR may not be the most optimal procedure for amplifying *Schistosoma* DNA in all geographic settings. Other procedures have been described and some of them seem to show promising diagnostic performance.<sup>26,27</sup> However, not all of these PCRs have been transformed into a multiplex real-time PCR format. Thus, they are substantially more laborious, still observer-dependent, and lack the option to include a proper internal control.<sup>27</sup> Also, some need relatively large serum volumes.<sup>28</sup> Thus, their feasibility for large-scale population based surveys still needs to be fully explored.

Human errors, including missed observations during microscopy and mislabeling and swapping of samples, can never be completely excluded. This problem is illustrated by the single sample showing > 50 eggs/10 mL of urine that showed negative results when tested by PCR. This person showed *S. haematobium* eggs at all six microscopy examinations, almost all with high egg counts. Thus, it is likely that aliquots were swapped during sampling for PCR analysis. The potential influence of human error is also illustrated by the performance of the real-time PCR at the separate laboratory facilities. Overall results were highly reproducible, but in case of discrepancy, the output produced by using an automated liquid handling station has been more in concordance with the microscopy data than the PCR output produced manually.

The use of an automated liquid handling station also enables processing of large sample numbers in a short period (i.e., we completed DNA isolation and detection, including automated data handling, for all 708 samples within 10 working days). The small sample volume required and the potential number of additional DNA targets that can be quantified, in particular when working with fecal samples, makes this approach highly attractive for large-scale population screening for the presence of multiple urinary and intestinal microorganisms.<sup>17,29,30</sup> In this study, we also introduced arbitrary units (AU) of copy numbers of *Schistosoma* DNA as PCR output, which provides a more realistic view on the existing high linear range of intensity of *Schistosoma* infection than  $C_t$  values. In addition, for more in depth comparison between different PCRs, further standardization, including converting DNA loads into egg counts, is needed.

In a previous study, the overall association between urine egg excretion and reported urinary and gynecologic symptoms was described. In particular, bloody discharge and a burning

sensation in the genitals were found to be significantly correlated with repeated urine microscopy findings, even after controlling for confounders.<sup>13</sup> As expected, we found the same associations between PCR-diagnosed schistosomiasis and reported genital symptoms. Because of the young age of the girls included, we were not able to collect any gynecologic samples, which would be of interest for quantification of *Schistosoma* DNA levels.<sup>31</sup> However, for confirmation, urinary and vaginal samples should be collected from adult women.

Although schools were randomly selected from a relatively small rural area of KwaZulu-Natal that had comparable ecologic and socioeconomic circumstances, we found *S. haematobium* infection prevalence to range from < 5% up to approximately 60% between the different schools. *Schistosoma* prevalence and the prevalence of reported urinary and gynecologic symptoms varied greatly between included schools. Conversely, we found no association between household composition, reflecting social status in this particular region, and the prevalence of *S. haematobium*. Focal distribution of schistosomiasis has been described and besides behavioral components in relation to exposure to infected water bodies, many other factors may influence prevalence and intensity of infection, including micro-level environmental circumstances.<sup>32</sup> The consequences are obvious. Although local hot-spots require intensive and repeated mass treatment with appropriate anti-schistosomal drugs in combination with intense control measures and monitoring, other communities will be served sufficiently by targeted treatment or even passive case detection. Based on this knowledge, there is also a clear need for a rapid and field applicable diagnostic test to allocate these hot spots in resource-poor settings without the need of high technology laboratory equipment.<sup>33,34</sup>

In conclusion, our findings illustrate that the diagnostic potentials of urine *Schistosoma* real-time PCR is dependent on the quality and intensity of performed microscopy. Repeated microscopy may in certain settings be more sensitive but at the same time it is a highly laborious as well as observer dependent procedure. Although improved sensitivity is still anticipated, parasite-specific DNA detection already seems an attractive and efficient automated high-output system for large screening programs. Only small sample volumes are needed to identify communities at risk for development of genital morbidity caused by *S. haematobium* infection.

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