Schistosoma haematobium Egg Excretion does not Increase after Exercise: Implications for Diagnostic Testing

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Abstract. Children are frequently invited to exercise before micturition, as it is believed that this activity will result in higher *Schistosoma haematobium* egg excretion, and hence, increases sensitivity of microscopic diagnoses. However, the evidence of this recommendation is scant. In the study presented here, 257 children, aged 2–15 years from south Côte d'Ivoire, provided urine samples for microscopy on consecutive days; one sample without prior exercise and one sample after exercise. Comparing the same individuals without and with prior exercise, sample positivity for *S. haematobium* (25.7% versus 23.0%, $P = 0.31$) and mean egg counts (10.2 eggs/10 mL versus 8.5 eggs/10 mL, $P = 0.45$) did not differ. Exercise before urine collection does not appear to increase *S. haematobium* egg excretion.

INTRODUCTION

Schistosomiasis is a neglected tropical disease affecting roughly 250 million people worldwide, with African settings representing 90% of the global burden of this disease.^{1,2} *Schistosoma haematobium*, one of the three main species causing schistosomiasis, affects the genitourinary system and is associated with considerable morbidity from pelvic pain, hematuria, anemia, infertility,^{3,4} the potential for increased risk of human immunodeficiency virus acquisition,⁵ and mortality from bladder cancer.^{4,6} The diagnosis of *S. haematobium* typically involves directly visualizing eggs by microscopy through standardized procedures^{7,8} or indirectly via evaluating individuals for hematuria.^{9,10} Both methods lack sensitivity, particularly when infection intensities are low. More sensitive diagnostic techniques for *S. haematobium* exist, such as serology, polymerase chain reaction (PCR), and schistosome circulating anodic antigen (CAA) testing,^{11–13} but these require sophisticated laboratory equipment, which are usually not widely available in resource-constrained settings.

Children in *S. haematobium*-endemic countries are frequently invited to perform a brief bout of exercise, such as jumping jacks, hopping up and down, or running up and down stairs before providing a urine specimen for the detection of *S. haematobium* eggs using microscopy (Figure 1). The underlying rationale is that such exercise results in higher egg excretion in the urine, and hence, may increase diagnostic sensitivity of microscopy. Indeed, this practice is embedded in the World Health Organization (WHO) guidelines,¹⁴ which state that “[p]hysical exercise combined with fluid intake has been shown to significantly increase egg output; it is therefore useful to ask the schoolchildren to do some short physical exercise before collecting the urine specimens”. However, the evidence based on this recommendation is

scant. We sought to evaluate the utility of physical exercise before urine collection on *S. haematobium* egg excretion in a community-based study in a primarily rural area of Côte d'Ivoire.

METHODS

We performed a cross-sectional study in six villages in the Azaguié region, south Côte d'Ivoire, located about 40 km north of the country's economic capital Abidjan. According to a recent national school-based survey, *S. haematobium* affects an estimated 5.3% of children in Côte d'Ivoire.¹⁵ The current research was conducted in August 2015 as part of a larger epidemiologic study. The purpose and procedures of the study were explained to the village and local health authorities. Ethical approval was obtained from the Ministry of Health and Public Hygiene of Côte d'Ivoire (CNER; reference no. 037/MSLS/CNER-dkn, approved July 22, 2015). Written informed consent from parents or guardians and oral assent from children was obtained before enrollment. All data were coded and entered into an Excel folder (Microsoft Corp., Redmond, WA). After completion of the study, all participants received a single 40 mg/kg oral dose of praziquantel at no cost, as per the national protocol.

Overall, 329 children aged 2–15 years (mean 6.8 years) were invited to provide midstream urine samples on two consecutive days. Urine was collected between 10:00 and 14:00 hours and processed by the filtration technique, adhering to standard protocols.^{7,8} Children were asked to provide a urine sample without prior exercise on the first day of the study and after exercise on the second day of the study. Exercise consisted of performing 20 “jumping jacks” for children aged 5 years and above. Children under the age of 5 years would have a parent or guardian holding them and gently lifting them up and down 20 times to mimic a jumping or bouncing motion. This exercise was conducted no earlier than 10:00 hours and the subsequent urine sample was collected.

Urine samples were transferred to a regional laboratory and processed the same day. In brief, 10 mL of urine was extracted

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FIGURE 1. Children performing exercise before providing a urine sample by running up and down a ramp, in the Azaguié region, Côte d'Ivoire. This figure appears in color at www.ajtmh.org.

from each urine sample after vigorously shaking the container. The extracted urine was pressed through a mesh filter, 13 mm in diameter, with 20 μ m pores (Sefar AG, Heiden, Switzerland). A drop of Lugol's iodine solution was placed over the sample before microscopy. Slides were examined under 10 $\times$ and 40 $\times$ lenses (Olympus Schweiz AG, Volketswil, Switzerland) and the presence, and if applicable, the number of *S. haematobium* eggs was recorded. For quality control, 10% of all slides were reexamined by a senior laboratory technician. These procedures were conducted on both days of the study. McNemar's test was used to evaluate test positivity between individuals on days 1 and 2, and a Wilcoxon signed-rank and paired *t* tests were used to compare egg counts in individuals with and without exercise.

RESULTS

Of the 329 children invited to participate in the study, 257 (78.1%) provided a urine sample on both days and were included in the final analysis. There were more males ($N = 157$, 61.1%) and most of the children were over 5 years of age ($N = 163$, 63.4%). On day 1 (without exercise), 66 of 257 samples (25.7%) were *S. haematobium*-positive, whereas on day 2 (after exercise), 59 of 257 (23.0%) were found positive (McNemar's *P* value: 0.31). Exercise before micturition did not increase mean egg counts in paired *t* tests comparing all children ($P = 0.45$), children aged < 5 years ($P = 0.31$), children aged 5–15 years ($P = 0.80$), among females ($P = 0.92$), or only

among males ($P = 0.19$) (Table 1). The Wilcoxon signed-rank testing yielded similar *P* values, with none below 0.1. Figure 2 shows paired egg counts for individuals with and without exercise before urine collection.

DISCUSSION

Schistosomiasis continues to be a major cause of morbidity in Africa despite ongoing efforts to curb this infection.¹⁶ Innovations to increase diagnostic sensitivity of existing tests, especially in low-intensity infections, are necessary for regional control and elimination strategies.^{8,17} Highly sensitive diagnostic tools, such as serologic assays, PCR testing, and CAA detection, are unfortunately not yet widely available for control and elimination programs in the most affected regions.^{11–13,18} Simple, effective, and inexpensive methods that can be used at the point-of-care with high diagnostic sensitivity would be very useful in the interim, whereas newer diagnostic methods are slowly implemented in endemic settings. Indeed, the discovery of circadian variations of *S. haematobium* egg excretion in the urine provides an example of how this biologic trait can be harnessed to increase diagnostic sensitivity,^{19–21} resulting in large-scale policy changes on how and when (i.e., mid-day) urine is collected.¹⁴ Exercise before micturition has been included in guidelines as a potential method of increasing *S. haematobium* egg excretion.¹⁴ However, to our knowledge, the one published study evaluating the relationship between exercise and urine egg excretion included only seven school-aged boys, four of whom conducted exercise in addition to ingesting 350 mL of fluid before providing a urine sample. This was part of a larger study involving 24 school-aged boys evaluating day-to-day and circadian variation in *S. haematobium* egg output in urine.²⁰ This study demonstrated that the four children who exercised had greater egg outputs compared with three children who did not, at different times of the day. Exercise in this study consisted of running 800 m and conducting 10 push-ups, 45 minutes before providing a urine sample. Our study did not find any association between exercise before micturition and *S. haematobium* egg output in a sample population much larger than the prior study (257 children with complete data records). In addition, we did not find any association between *S. haematobium* egg outputs after exercise in different age groups or sexes. Our findings suggest that exercise before micturition does not increase *S. haematobium* egg outputs in urine and may not be a useful recommendation to increase diagnostic sensitivity.

There are potential weaknesses of this study that may contribute to not finding an association between exercise and

TABLE 1
Results of paired *t* tests, comparing *Schistosoma haematobium* egg excretion with or without jumping before providing a urine sample

Demographic	<i>N</i> (%)	Mean egg concentration* (no exercise)	Mean egg concentration* (with exercise)	<i>P</i> value
All ages	257 (100)	10.2	8.5	0.45
Age 2–4 years	94 (36.6)	6.4	3.1	0.31
Age 5–15 years	163 (63.4)	12.4	11.7	0.80
Female	100 (38.9)	8.8	9.2	0.92
Male	157 (61.1)	11.1	8.1	0.19

* Egg concentration, defined as eggs per 10 mL of urine.

FIGURE 2. Measured urine *Schistosoma haematobium* egg concentration for the same individuals with and without prior exercise.

S. haematobium egg excretion in urine. First, to isolate the effects of exercise, we did not specifically promote 350 mL of fluid intake before micturition as was done by Doehring et al.²⁰ In our collective experience, fluid ingestion before micturition is not a widely used practice to increase *S. haematobium* egg excretion, and hence, was not included in our protocol. Second, the exercise conducted in our study consisted of 20 jumping jacks (or 20 gentle “bounces” in those under 5 years of age), which may be less vigorous compared with the exercise conducted in prior studies. Perhaps greater exercise intensity could result in greater *S. haematobium* egg excretion. Last, our study design would be stronger had we used a randomized cross-over block design, such that children would provide urine samples on two consecutive days, but some children would exercise on the first day and others would exercise on the second day. The reason this protocol was not used (as was our initial intention) was due to concerns based on earlier observations that when some children performed exercise, then others would as well, even if they were not randomized to do so. Such deviation from randomization could mask any potential impact of our intervention.

Taken together, exercise before micturition is a common behavioral practice in African settings as it is believed to help increase *S. haematobium* egg excretion and diagnostic sensitivity of microscopy, and hence, is being recommended in the WHO guidelines.¹⁴ We did not find an association between *S. haematobium* egg excretion and exercise in this study and doubt whether this practice improves the sensitivity of *S. haematobium* diagnoses. However, future studies evaluating the link between exercise and *S. haematobium* egg excretion may be required to draw definitive conclusions.

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REFERENCES

- Colley DG, Bustinduy AL, Secor WE, King CH, 2014. Human schistosomiasis. *Lancet* 383: 2253–2264.
- Hotez PJ et al., 2014. The Global Burden of Disease study 2010: interpretation and implications for the neglected tropical diseases. *PLoS Negl Trop Dis* 8: e2865.
- Koukounari A, Gabrielli AF, Touré S, Bosqué-Oliva E, Zhang Y, Sellin B, Donnelly CA, Fenwick A, Webster JP, 2007. *Schistosoma haematobium* infection and morbidity before and after large-scale administration of praziquantel in Burkina Faso. *J Infect Dis* 196: 659–669.
- van der Werf MJ, de Vlas SJ, Brooker S, Looman CWN, Nagelkerke NJD, Habbema JDF, Engels D, 2003. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta Trop* 86: 125–139.
- Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, Downs JA, 2011. Examining the relationship between urogenital schistosomiasis and HIV infection. *PLoS Negl Trop Dis* 5: e1396.
- El-Bolkainy MN, Mokhtar NM, Ghoneim MA, Hussein MH, 1981. The impact of schistosomiasis on the pathology of bladder carcinoma. *Cancer* 48: 2643–2648.
- WHO, 2002. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee. *WHO Tech Rep Ser* 912: 1–57.
- Utzinger J, Becker SL, van Lieshout L, van Dam GJ, Knopp S, 2015. New diagnostic tools in schistosomiasis. *Clin Microbiol Infect* 21: 529–542.
- Bogoch II, Andrews JR, Dadzie Ephraim RK, Utzinger J, 2012. Simple questionnaire and urine reagent strips compared to microscopy for the diagnosis of *Schistosoma haematobium* in a community in northern Ghana. *Trop Med Int Health* 17: 1217–1221.
- Mott KE, Dixon H, Osei-Tutu E, England EC, Ekue K, Tekle A, 1985. Evaluation of reagent strips in urine tests for detection of *Schistosoma haematobium* infection: a comparative study in Ghana and Zambia. *Bull World Health Organ* 63: 125–133.
- Bierman WF, Wetsteyn JC, van Gool T, 2005. Presentation and diagnosis of imported schistosomiasis: relevance of eosinophilia, microscopy for ova, and serology. *J Travel Med* 12: 9–13.
- Obeng BB et al., 2008. Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium* in urine samples from Ghana. *Ann Trop Med Parasitol* 102: 625–633.
- De Jonge N, Fillie YE, Hilberath GW, Krijger FW, Lengeler C, de Savigny DH, van Vliet NG, Deelder AM, 1989. Presence of the schistosome circulating anodic antigen (CAA) in urine of patients with *Schistosoma mansoni* or *S. haematobium* infections. *Am J Trop Med Hyg* 41: 563–569.
- WHO, 2011. *Helminth Control in School-Age Children: A Guide for Managers of Control Programmes*. Geneva, Switzerland: World Health Organization.
- Chammartin F et al., 2014. Bayesian risk mapping and model-based estimation of *Schistosoma haematobium*-*Schistosoma mansoni* co-distribution in Côte d'Ivoire. *PLoS Negl Trop Dis* 8: e3407.
- Lo NC et al., 2017. A call to strengthen the global strategy against schistosomiasis and soil-transmitted helminthiasis: the time is now. *Lancet Infect Dis* 17: e64–e69.
- Lo NC, Bogoch II, Blackburn BG, Raso G, N'Goran EK, Coulibaly JT, Becker SL, Abrams HB, Utzinger J, Andrews JR, 2015.

- Comparison of community-wide, integrated mass drug administration strategies for schistosomiasis and soil-transmitted helminthiasis: a cost-effectiveness modelling study. *Lancet Glob Health* 3: e629–e638.
18. Knopp S et al., 2015. Sensitivity and specificity of a urine circulating anodic antigen test for the diagnosis of *Schistosoma haematobium* in low endemic settings. *PLoS Negl Trop Dis* 9: e0003752.
  19. McMahon JE, 1976. Circadian rhythm in *Schistosoma haematobium* egg excretion. *Int J Parasitol* 6: 373–377.
  20. Doebling E, Feldmeier H, Daffalla AA, 1983. Day-to-day variation and circadian rhythm of egg excretion in urinary schistosomiasis in the Sudan. *Ann Trop Med Parasitol* 77: 587–594.
  21. Pugh RN, 1979. Periodicity of output of *Schistosoma haematobium* eggs in the urine. *Ann Trop Med Parasitol* 73: 89–90.