Seasonal variations in *Schistosoma haematobium* egg excretion in school-age girls in rural KwaZulu-Natal Province, South Africa

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Background. A predominant feature of *Schistosoma haematobium* infection is urinary egg excretion, and microscopic egg detection remains the accepted standard field diagnostic tool. Praziquantel is the drug of choice for schistosomiasis, and the World Health Organization recommends that it should be administered to all children >4 years of age living in schistosomiasis-endemic areas. The frequency of mass drug administration depends on the prevalence rate in the community. Urinary schistosome egg output has a day-to-day and hour-to-hour intrasubject variation. Therefore, it is important to assess possible seasonal variations in egg excretion to improve the planning of drug treatment.
Objectives. To assess the influence of seasonality on urinary schistosome egg excretion in South Africa (SA).
Methods. We performed a prospective cohort study, exploring seasonal variations of *S. haematobium* egg excretion in 184 girls aged 10 - 12 years from randomly selected schools in a rural area of KwaZulu-Natal Province, SA. The area has a subtropical climate characterised by a cool dry season and a hot humid season. For children, water contact is higher in the latter season. At baseline, 108 girls were examined in the hot season, and 76 in the cold season. In the next year's cold season the untreated patients were re-investigated before treatment.
Results. There was a decrease in infection in the group initially tested in the hot season compared with the group tested in the cold season at both time points when adjusted for age and water contact (adjusted odds ratio 3.61 (95% confidence interval 1.14 - 11.44); p=0.03).
Conclusions. This unique study shows that schistosomiasis prevalence determined by microscopy exhibits seasonal variation, with a higher prevalence in the hot rainy season. Precise community prevalence estimations are key in decisions to treat communities. There was significantly lower egg output in the cold season, and sampling in that season may therefore underestimate the prevalence of urinary schistosomiasis. The study indicates that sampling in SA should be done in the hot season.

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Worldwide an estimated 218 million people have schistosomiasis, of whom ~90% live in Africa.[1] Schistosomiasis is a parasitic disease caused by *Schistosoma* trematodes.[1] It is endemic in KwaZulu-Natal (KZN) Province, South Africa (SA), and *Schistosoma haematobium* is the most common species.[2] *S. haematobium* causes urogenital schistosomiasis, with symptoms including stunting, infertility, cancer, hydronephrosis and increased susceptibility to HIV.[1,3] Other schistosome species, the most common being *S. mansoni* and *S. japonicum*,[3] are not endemic in KZN.[2] Mass drug administration with praziquantel is the primary tool for combating schistosomiasis, and school-age children are the main target group. The frequency of mass drug administration depends on a community's precontrol prevalence rate, which is assessed by parasitological methods or questionnaires, differentiating into low (<10%), moderate (10 - 50%) and high risk (>50%) communities.[4] Egg counts by microscopic examination of urine remain the accepted standard diagnostic tool for detecting *S. haematobium* infection.[5,6] When egg excretion is low, the sensitivity of urine microscopy for ova is poor. Consequently, communities' need for intervention might be underestimated.[3]

There are seasonal variations in the transmission of schistosomiasis in infested water sources,[7] also in SA.[2,8] Furthermore, there are intrasubject variations in *S. haematobium* egg output.[9-11] A few, albeit small, studies indicate that egg excretion may vary, depending on the season, but none of these has been done in a country with a subtropical climate, such as SA.[12-14] As community prevalence is the basis for decisions to treat, it is important to identify the optimal period for assessment of egg excretion and community prevalence. In anticipation of planned control programmes in SA, the objective of this observational study was to assess the influence of season on urinary schistosome egg excretion.

Methods

Study population, area and climate

The study was conducted in the rural inland Ugu District, SA. In 2011, 16.1% of the district's total population did not have access to piped water, and people relied on unsafe water from nearby rivers.[15] The area has a subtropical climate, characterised by hot and humid summers (December through April), and dry and cool winters (June through August) (Fig. 1).[16,17] For children, water contact is higher in summer, especially from mid-December to mid-January, which is a school holiday period in this region.[17]

Study inclusion and sampling

In 2010, baseline data were collected from girls aged 10 - 12 years, who were attending 18 randomly selected schools. The schools were visited in a random fashion. All girls 10 - 12 years of age were invited to take part in the study, and 1 057 gave consent (Fig. 2). This cohort was initially selected for studies on female genital schistosomiasis and hence no boys were recruited. From February to April (hot season) and June to November (cold season), 970 of these girls submitted a urine sample (omitting December, January and May owing to school holidays, examinations or examination-related activities).[18] A total of 940 participants had reliable age data (Fig. 2). Only one case of *S. mansoni* was found at baseline.[18]

In 2011, all 18 schools were revisited in the cold season (from end of May to September). Nurses from the Department of Health offered a single dose of praziquantel 40 mg per kg of body weight.[15] For research purposes, every third pupil was asked for a urine sample on the same day. One school did not provide urine samples. Some participants could not be found and some did not provide urine samples (Fig. 2). In total, 184 of the participants submitted urine samples at follow-up, which were collected in the cold season.

Of the 184 participants who submitted a urine sample at two time points, baseline urine specimens from a subgroup were collected in the hot season (February - April; group 1, *n*=108) or cold season (June - November; group 2, *n*=76) for follow-up later in the cold season (Fig. 2).

At baseline, the participants were interviewed face-to-face in isiZulu on water contact, schistosomiasis infection (local name: *isichenene*) and if they had received prior treatment. All urine specimens were collected between 10h00 and 14h00 and all were processed by the same method as described previously.[18] The participants were not given fluids before sampling.

Data management and statistical analyses

Data were entered into Excel (Microsoft Corp., USA) and subsequently imported into SPSS Version 22 (IBM Corp., USA), using two-pass verification for quality control. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to compare schistosomiasis prevalence, previous water contact or treatment in groups 1 and 2. The Mann-Whitney *U*-test was used to compare the duration between baseline and follow-up, because of the non-normally distributed continuous data. For normally distributed continuous data (age), Student's *t*-test was used. For paired data (baseline and follow-up), the Wilcoxon signed-rank test was used for non-normally distributed continuous data (urine *S. haematobium* intensity) and McNemar's test for nominal data (prevalence). To study the impact of several variables simultaneously, logistic regression analysis was applied at a 5% significance level; variables were included if the *p*-value from crude association was <0.2 and if the Spearman rank correlation coefficient was <0.7.

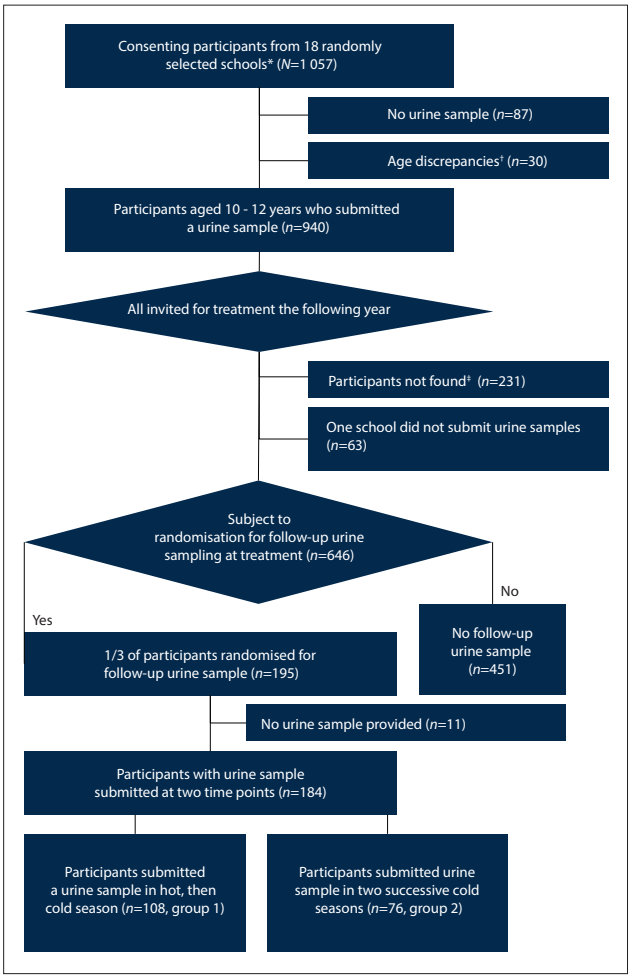

*Fig. 2. Flowchart showing selection and randomisation of participants. (*Only girls, as this was part of a study on female genital schistosomiasis. †Provided irreconcilable dates of birth and were found to be up to 4 years older by the new dates provided. These were excluded as urinary schistosomiasis egg excretion is age-dependent.[19] ‡Teachers/peers were not asked about reasons for the participants' absence.)*

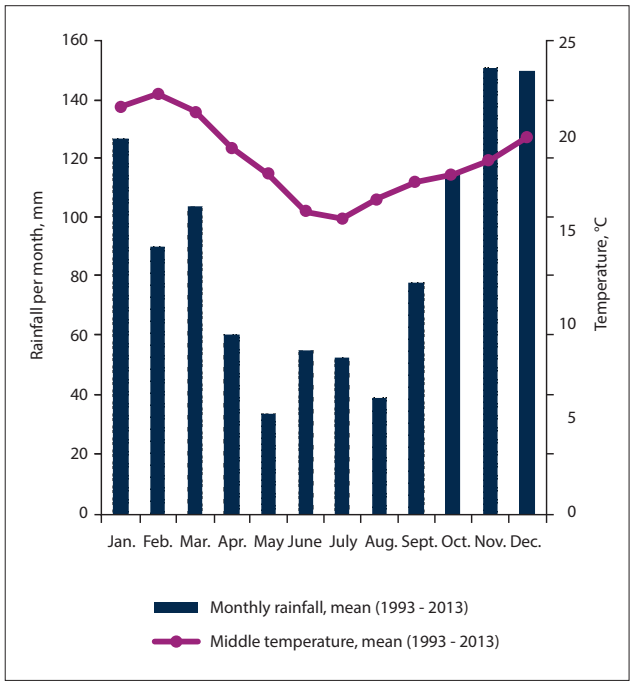

Fig 1. Meteorological registrations, Paddock weather station (nearest weather station).[16]

Ethical approval

The project was approved by the Biomedical Research Ethics Committee, University of KwaZulu-Natal (ref. no. BF029/07), the Department of Health, Pietermaritzburg (ref. no. HRKM010-08), the Norwegian Regional Committee for Medical and Health Research Ethics (ref. no. 469-07066a1.2007.535) and the European Group on

Ethics in Science and New Technologies (ref. no. IRSES-2010:269245). The departments of Health and Education in Ugu District granted permission.[15,18] The participants were included only if a guardian gave written consent and the participant assented. Antihelminthic treatment was delayed owing to lack of access to praziquantel.[20] As soon as praziquantel could be accessed, treatment was offered free by the Department of Health. The medical consequences of the delay are unknown.

Results

General characteristics

A total of 940 girls aged 10 - 12 years submitted urine samples. A median of 42 (range 7 - 171) girls were included from each of the 18 schools. The mean age was 11.1 (standard deviation (SD) 0.8) years. At baseline, 27.6% (259/940) were *S. haematobium*-positive, and in the positive group the geometric mean count was 23 eggs/10 mL urine.

Follow-up study

Of the girls who were examined at baseline, 184 were re-examined during the following year's cold season. The median interval between the examinations was 14.8 (7.3 - 15.6) months. There had been no mass treatment in the interim.

Urine specimens from 108 participants were initially submitted in the hot season of 2010, with second urine specimens in the cold season of the following year (Table 1, group 1). Moreover, urine samples from 76 participants collected in the cold season of 2010 (group 2) were compared with a second urine specimen collected during the next cold season. Thus, group 2 submitted urine specimens in two cold seasons. There was no significant difference in age between the two groups (Student's *t*-test, $p=0.662$). The interval period was somewhat longer for the participants tested in the hot season at baseline (group 1, median 15.3 (14.3 - 15.7) months) than for those tested in the cold season at baseline (group 2, median 12.0 (7.3 - 12.1) months; Mann-Whitney *U*-test, $p<0.001$).

At follow-up, 22.3% (41/184) were *S. haematobium*-positive and the geometric mean count was 14 eggs/10 mL urine. In group 1 (participants tested initially in the hot season, then in the cold season), the intensity of urinary schistosomiasis decreased significantly (Wilcoxon signed-rank test, $p=0.05$). In group 1, the prevalence at baseline was 38% (41/108) and at follow-up 29% (32/108) (McNemar test, $p=0.09$). The baseline and follow-up prevalences of group 2 (tested in the cold season on both occasions) did not differ significantly (McNemar test, $p=0.76$) – likewise for urinary schistosomiasis intensities (Wilcoxon signed-rank test, $p=0.39$) (Table 1).

Interestingly, as shown in Fig. 3, there was no significant difference in conversion to positive schistosomiasis status in the groups after adjusting for age and water contact (adjusted OR 1.77 (95% CI 0.47 - 6.67); $p=0.40$). Conversion to negative schistosomiasis status was more common in those who were examined in the hot season initially than in those who were tested in the cold seasons both times (adjusted OR 3.61 (95% CI 1.14 - 11.44); $p=0.03$). The girls who were tested in the hot season initially had a higher schistosomiasis prevalence, more water contact, and more self-reported schistosomiasis (Table 1).

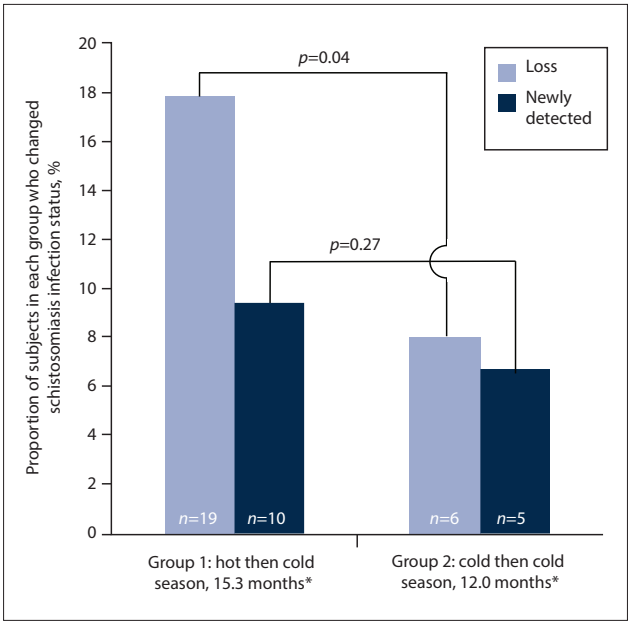

Fig. 3. Proportion of subjects who changed Schistosoma haematobium *infection status between baseline and follow-up. (*Median duration between baseline and follow-up.)*

Table 1. Comparison of schistosomiasis in two groups of girls aged 10 - 12 years in Ugu District, KwaZulu-Natal, South Africa

	Baseline urine collected in hot season (group 1)	Baseline urine collected in cold season (group 2)	OR (95% CI)	*p*-value
Participants, *n* (%)	108 (58.7)	76 (41.3)	-	-
Schools, *n*	10	7	-	-
Reported having had schistosomiasis,* *n* (%)	36/105 (33.3)	9/73 (11.8)	3.7 (1.7 - 8.3)	0.001
Reported prior treatment for schistosomiasis,* *n* (%)	20/108 (18.5)	4/72 (5.3)	4.1 (1.3 - 12.5)	0.014
Reported freshwater contact,*† *n* (%)	90/108 (83.3)	26/76 (34.2)	9.6 (4.8 - 19.2)	<0.001
Prevalence, %				
Baseline	38.0	13.2	4.0 (1.9 - 8.7)	<0.001
Follow-up‡	29.6	11.8	3.1 (1.1 - 7.0)	0.006
Intensity (eggs/10 mL)§				
Baseline	18.4	9.0	1.6 (1.2 - 2.1)	0.001
Follow-up‡	14.9	12.9	1.4 (1.1 - 1.9)	0.012

OR = odds ratio; CI = confidence interval.
*Interviewed at baseline, the isiZulu term *isichenene* was used for schistosomiasis.
†Water contact is defined as any water contact (river/dam/lake/stream) at any time.
‡All follow-up urine samples were collected in the cold season. There was no treatment in the time period between baseline and follow-up. Denominators in the table vary because of missing values.
§Geometric mean. Regression analysis using log-transformed values.

Discussion

In this novel study of untreated children over a period of >1 year in rural SA, we found that the *S. haematobium* prevalences and intensities were lower in the cold season than in the hot season. This may indicate that urine sampling in the hot season in this subtropical climate yields higher prevalences and intensities, which could have an impact on the decision to provide mass treatment.

Seasonal water contact patterns and snail density fluctuations may affect the transmission rate of *S. haematobium,* but it is not known if ovum excretion has a parallel presentation.[7,12,14] The worm lives for a mean period of 5 years; there are also numerous reports of live ova excretion many years after exposure, indicating worm longevity and persistent ability to lay eggs.[3] Low prevalence and intensity in the cold season are more likely to be caused by a lower ability to lay eggs rather than a number of worms dying during the first year of infection. Evolution of antifecundity immunity during the cold season is a possible source for reduction in the observed egg excretion.[21] If this is the case, the detection rates might be highest in the weeks after worm maturation, ~6 weeks after infection. The low ova excretion and thus lower microscopy sensitivity may lead to a lower observed prevalence. The subtropical climate of SA differs from most areas usually endemic of schistosomiasis, as it has a longer cold season with less water contact. We would argue that a long cold season would probably decrease the observed prevalence more than a shorter season with low water contact.

While polymerase chain reaction-based assays and circulating anodic antigen (CAA) tests are both more sensitive alternatives to urine microscopy, they are either too costly in a field context or not available commercially.[6,22] Therefore, urine microscopy for *S. haematobium* ova continues to be a preferred field diagnostic test. However, our results indicate that urine microscopy should be done during the hot season in SA, ensuring as little underestimation of prevalence as possible.

Repeated investigations without treatment are usually not done, as it is unethical to leave people untreated. However, a few other, albeit small, studies indicate that egg excretion may vary through the seasons of a year.[12,13,23,24] We can possibly preclude a decrease in age-dependent schistosomiasis prevalence, as the age of peak prevalence is expected later in moderate endemic areas.[19,25] We cannot preclude that some participants might have sought treatment between the two examinations, although only 15% reported having received such treatment before baseline in local clinics.[18] We also cannot preclude that the participants were dehydrated in the hot season and therefore yielded higher egg outputs, although water was not offered at any sampling. Furthermore, the two groups are different, even though they were visited in a random sequence.

Conclusions

This study indicates that microscopy of urine collected in the cold season might cause an underestimation of the prevalence in SA. This would lead to an underestimation of the need for treatment. In the current absence of more sensitive field diagnostics for *S. haematobium*, the use of urine microscopy remains the standard field diagnostic test. To ensure correct prevalence rates and correct interventions in schools, we cautiously suggest that the best time for collection of urine samples is the hot season. Further research is needed to explore if the situation is similar in boys and in different climatic areas.

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Author contributions. EEC, EFK, SGG and MT designed the study. EEC, SH and EFK analysed the data. EEC, SGZ, EK, EFK and KL collected the data. EEC wrote the original manuscript and managed the database. MT, SGZ, KL, SGG, SH, EK, BJV, PDN and EFK reviewed and edited the manuscript.

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Conflicts of interest. None.

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