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The role of hygienic bathing after defecation in the transmission of *Schistosoma mansoni*

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Summary Transmission of *Schistosoma mansoni* depends on fecal eggs reaching water, but the way this happens is poorly understood. We studied the role of hygienic bathing after defecation in the contamination of water with *S. mansoni* eggs. Individuals in an endemic community in Northern Senegal ($n = 991$) were examined for *S. mansoni* infection and a random sample (22%) was interviewed about stool disposal practices and hygienic behavior. We assessed the presence and viability of *S. mansoni* eggs adhering to the peri-anal region of 13 infected volunteers, by counting the miracidia in the water they had used for hygienic washing; for 10 of them (77%) miracidia were demonstrated. From the population infection distribution, average number of defecations per day, proportion of individuals bathing after defecation, and association between miracidial counts and infection intensity, we calculated a daily population miracidial output of ~30 000 through hygienic bathing. For comparison, one complete stool reaching the water was calculated to yield ~2500 miracidia. Thus, 12 individuals in this population should defecate into the water every day to produce the same number of miracidia as through hygienic bathing. Our results suggest a major role of hygienic bathing after defecation in the transmission of *S. mansoni*.

1. Introduction

Transmission of *Schistosoma mansoni*, a parasitic worm responsible for intestinal schistosomiasis, depends on viable eggs in human excreta reaching water inhabited by snail

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intermediate hosts. The way in which this happens is probably the least understood part of the life cycle of the parasite. For urinary schistosomiasis, caused by *S. haematobium*, it is conceivable that children in particular but also adults urinate directly into the water. For intestinal schistosomiasis, however, direct defecation into the water seems much less likely. In fact, the act of defecation is a cultural taboo in many traditional societies, and direct deposition of stools into streams is considered unacceptable, even for children (Chandiwana, 1986; Curtis et al., 1993). Nevertheless, *S. mansoni* transmission is very efficient, as appears from the high re-infection rates that are commonly observed after treatment programs (Gryseels, 1996).

Many have favored the hypothesis that infected stools deposited on the banks of rivers and ponds are washed into the water by heavy rains or floods (Vercruysse et al., 2001). This undoubtedly occurs, but can account for transmission to humans only during the rainy or post-rainy seasons, given the limited life span of infected snails (Anderson and May, 1979; Sturrock et al., 1979; Theron and Coustau, 2005). Nevertheless, infection and re-infection is known to be rather common in the dry season (Chandiwana, 1986; Sturrock et al., 2001). Another possible, although rather coincidental, cause of contamination of water with *S. mansoni* eggs is by animals (e.g. cattle, dogs) walking through defecation sites and carrying the human feces on their hooves or legs into the water. Animals may also act as reservoir hosts of human schistosomiasis and as such contribute to the transmission cycle, as suggested by different reports on natural infections with *S. mansoni* in rodents (Mansour, 1973; Rollinson et al., 1986). However, the role of animal reservoirs in *S. mansoni* transmission is usually considered to be modest or even nonexistent (Adewunmi et al., 1991; Duplantier and Sene, 2000).

We hypothesize that bathing after defecation (with small amounts of fecal material with viable schistosome eggs adhering to the peri-anal region being washed into the water) plays an important role in the transmission of *S. mansoni*. Transmission of this type (henceforth indicated as 'hygienic bathing' or 'hygienic washing') is expected to be year-round and does not depend on a series of accidental events. The aim of this study in Northern Senegal was to determine the presence and viability of *S. mansoni* eggs trapped in the peri-anal region of infected individuals, and to estimate the relative importance of hygienic bathing in the contamination of water with *S. mansoni* eggs.

2. Materials and methods

2.1. Study population

This study was performed in September 2003 in Thiago, a village of about 1200 inhabitants in Northern Senegal, endemic for *S. mansoni*, close to a stream and with a school and a functional community health center. A study on defecating behavior and hygienic practices in children in the same area showed that most of them never or rarely visited latrines, but defecated somewhere else (Sow et al., 2004). A popular place appeared to be in nature, in particular near streams. This was confirmed by observations based on mapping of defecation sites, which showed a considerable number of stools just a few meters from the riverbank. Most children reported using the water to clean themselves after defecating (i.e. hygienic bathing).

For all individuals of 5 years and above who agreed to participate in this study after informed consent, the intensity of *S. mansoni* infection was determined based on the number of schistosome eggs counted in a duplicate 25 mg Kato–Katz smear of two stools (Katz et al., 1972). Subsequently, a number of sub-studies were conducted to provide an estimate of the overall population miracidial production per day through the habit of hygienic bathing after defecation, compared to occasional direct defecation into the stream.

2.2. Data collection

First, a group of 13 volunteers (eight boys and five girls, all between 5 and 20 years old) with relatively high egg counts agreed to participate in the study to determine the presence and viability of parasite eggs in the peri-anal region, providing a measure of contamination through hygienic washing in the stream after defecation. Each participant received a kettle filled with 35 cl of 0.85% saline spring water and a plastic bucket. Instead of using water from the stream to wash after defecation, they were asked to use the provided saline water, collect the water with fecal remnants in the bucket and bring it to the laboratory. The water was then tested for the presence of miracidia (i.e. the developing larvae in the *Schistosoma* eggs) by a hatching test, and the number of miracidia per individual was counted.

A second group of 12 volunteers (nine boys and three girls, 5 to 20 years old) was willing to test the hatchability of eggs in fresh stools, providing a measure of contamination through occasional direct deposition of stools into the stream. For that purpose, each participant was asked to defecate in private and then bring the complete stool to the lab as soon as possible (which was always within 10 min). After homogenization, a sample of 2 g was taken from each fresh specimen for the hatching test.

2.3. Sample analysis

The number of viable miracidia for both groups was determined by a slightly adapted hatching procedure (Cheever, 1978; Upatham et al., 1976) (Tchuem Tchuente, personal communication). The samples were flushed and, for the second group, pressed through a nylon 500 μm tea strainer above one or more cone-shaped plastic cups of 100 ml. The strainer was rinsed with 0.85% saline spring water. The cups were left for sedimentation for 20 min. The supernatant was discarded, and saline water was added again. This procedure was repeated three times until the water cleared. Spring water was added to the sediment and poured into a side-armed flask. Water was added to the flask up to the brim. The flask was then covered with aluminum paper, except for the top of the side arm (2 cm), which was illuminated with a cold light torch. With a Pasteur pipette, approximately 1 ml of the supernatant was picked up after half an hour of exposition and directly placed into a cell well and checked for miracidia with a binocular microscope. This was repeated every 20 min and continued until 5 h after the

start of the experiment. Samples that continued to show miracidia at the end of the day were re-checked the next morning. For each individual, the miracidial counts of all wells were aggregated and related to their egg load (eggs per gram, epg) as determined by Kato–Katz smear (see above).

Furthermore, a simple questionnaire was administered to a random sample of a quarter of the population about their defecating behavior. Questions included the average number of defecations per day and whether or not the interviewee practiced habitual bathing after defecation.

Finally, to provide crude estimates of average daily stool weights, five infected volunteers (two children and three adults) were asked to collect their 24 h stools for two consecutive days in a labeled plastic bucket (with cover) with a cellophane bag inside.

2.4. Statistical analysis

Linear regression was used to calculate the association between the log-transformed number of miracidia and the egg load, both for the group of 13 volunteers that provided anal wash water and the group of 12 volunteers that provided fresh stool samples.

From the above sub-studies, we calculated the overall population miracidial output through hygienic bathing and compared this value with the number of miracidia resulting from a complete stool reaching the water. The ratio between both values provides an estimate of the number of stools needed to be deposited into the water to equal the contribution from hygienic bathing to the contamination of water with *S. mansoni* eggs.

We applied univariate sensitivity analysis by varying the parameters underlying this ratio, including the 90% CI of the statistical associations between miracidial and egg counts. For the average daily stool weights, we used the median results of a bigger stool sample study on another parasitic worm disease in St Lucia as alternative values: 90 g (5–9 years), 122 g (10–14), 145 g (15–19), 160 g (20–29), 173 g (30–39) and 186 g (40 and above) (Bundy et al., 1987).

3. Results

Of the 991 inhabitants of Thiago (5 years and above) that agreed to participate in our study, 62% were found positive for *S. mansoni* infection. The overall mean intensity was 340 epg and did not show a marked pattern with age (Table 1). In total, 218 subjects (i.e. 22% of the study population) were interviewed about their defecating behavior. The average reported number of defecations per day was 1.6. This value ranged from 1.9 among the youngest age group to 1.2 among the oldest. On average, 47% reported practicing habitual bathing after defecation. This behavior occurred more often among children (52%) than in the oldest age group (33%). The average weight of 24 h stools was 130 g for the two children, and 194 g for the three adults.

Figure 1A shows the association between individual egg counts and miracidial output after hygienic washing for 13 volunteers. Ten out of the 13 samples (77%) produced miracidia. Eggs in the peri-anal region hatched not only for people with high egg counts, but also for those who had

Table 1 Calculation of the *Schistosoma mansoni* miracidial production for the village of Thiago, Northern Senegal, comparing contamination of the water through hygienic washing with (possible) direct deposition of stools

Age group (years)	Epidemiological study		Questionnaire study			Miracidial output (thousands)	
	N_E[a] (a)	Arithmetic mean epg (b)	N_Q[a]	No. defecations per day (c)	Hygienic washing after defecation (%) (d)	Through hygienic washing per day (e)[b]	Through direct deposition of one stool (f)[c]
5–9	300	257	70	1.9	51.4	9.0	1.1
10–14	193	289	40	1.6	53.0	5.7	1.5
15–19	168	423	25	1.4	48.0	5.7	3.8
20–29	118	638	42	1.6	42.4	6.1	5.0
30–39	89	133	20	1.3	41.6	0.8	1.3
≥40	123	373	21	1.2	33.4	2.2	3.9
Total	991	340	218	1.6	46.9	29.5	
					Weighed mean (all age groups):		2.5
					Weighed mean (5–14 years):		1.3

[a] N_E and N_Q represent the number of individuals in Thiago for the epidemiological and questionnaire study, respectively.
[b] The value of (e) = 0.12 (a).(b).(c).(d)/100, where 0.12 followed from Figure 1A.
[c] The value of (f) = (0.13/2).*W*.(b)/(c), where 0.13 followed from Figure 1B, and *W* is the weight of 24 h stools: i.e. on average 130 g for children (5–14 years) and 194 g for adults (≥15 years).

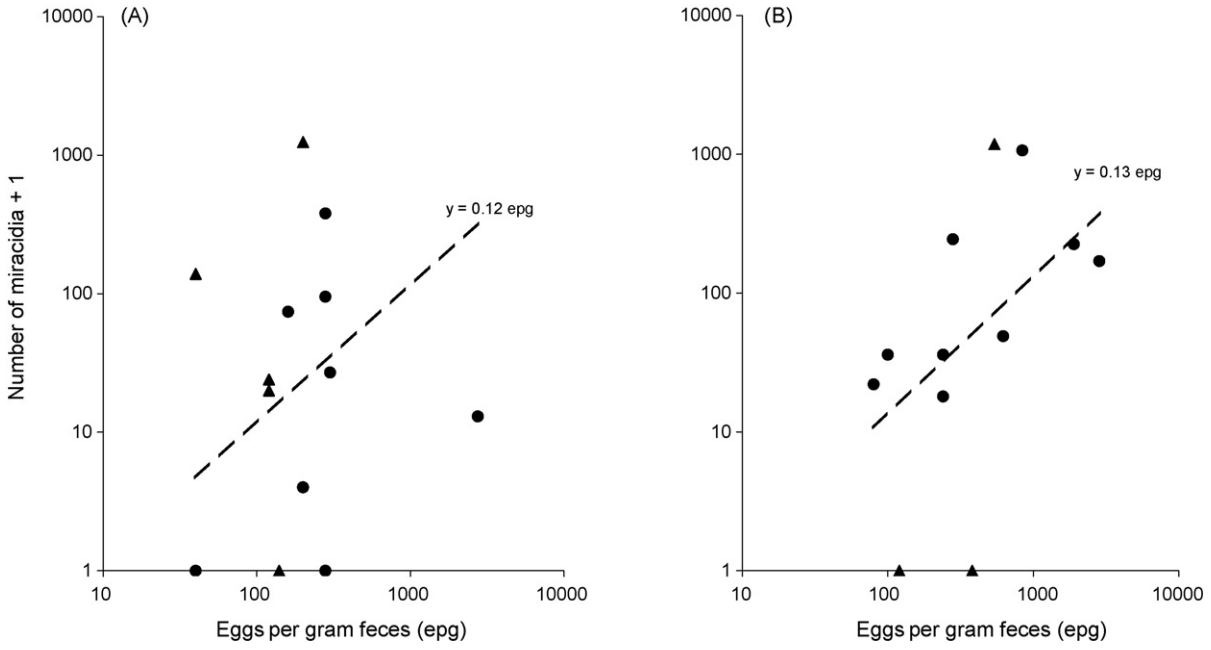

Figure 1 Association between number of miracidia, measured by hatching, and the intensity of *Schistosoma mansoni* infection, measured by Kato—Katz fecal smear. The fecal material for hatching came from: (A) hygienic washing by 13 volunteers and (B) random samples of 2 g fresh stool from 12 volunteers. Triangles and dots represent females and males, respectively. The curves were based on linear regression of log [number of miracidia + 1] against log [epg], assuming a proportional association.

relatively light infections. Linear regression of log [number of miracidia + 1] and log [epg] resulted in a slope that did not differ significantly from 1. Therefore, the overall association between miracidial count (y) and egg counts could be assumed to be proportional: $y = 0.12$ epg (90% CI 0.038—0.37; $P < 0.01$). The association between egg counts and miracidial counts for fresh stools showed less variability (Figure 1B). Here, 10 out of the 12 samples (83%) produced miracidia, and miracidial counts showed a clearly increasing trend with egg counts. Again, the overall statistical association could be assumed to be proportional: $y = 0.13$ epg (90% CI 0.052—0.34; $P < 0.01$). For both associations, age and sex of the host had no significant effect (data not shown).

From the values above, it can be calculated that this population of nearly 1000 individuals releases approximately 30 000 miracidia per day due to hygienic bathing (Table 1). Direct deposition of a single complete stool into the water would result in approximately 2500 miracidia. Thus, overall around 12 (= 30 000/2500) complete stools should reach the water every day in order to contribute to transmission in the same order of magnitude as hygienic bathing. For children (5—14 years), one complete stool would result in about 1300 miracidia, mainly due to the smaller size of their stools. In that case, as many as 23 stools would be needed to match up with the number of miracidia produced after hygienic washing.

Sensitivity analysis revealed that the variability of the statistical association between miracidial count and egg counts (Figure 1A) has the biggest impact; the 90% CI corresponds to a range of 4 to 37 complete stools, matching the population miracidial output through hygienic bathing. Using the daily stool production as observed in the study in St Lucia (Bundy et al., 1987) resulted in a slightly higher average (14 stools instead of 12).

4. Discussion

In the majority of the individuals infected with *S. mansoni*, we could demonstrate active miracidia in the water they used for hygienic washing. This confirms that the infection can indeed be transmitted through this route. The overall population miracidial output through hygienic bathing matched with not less than four and on average 12 complete stools deposited directly in the water every day. Given the common practice of using streams for hygienic washing in this population in Northern Senegal, it may thus play a major role in the continuous and efficient transmission of *S. mansoni*.

We appreciate that our calculations of population miracidial output are based on small numbers of subjects, which allow for semi-quantitative results at most. It was not possible to recruit more individuals on a voluntary basis. Nevertheless, our finding that hygienic bathing by a whole population results in considerably more miracidia than a single stool deposited in the water is robust to a range of alternative assumptions, as demonstrated by the sensitivity analysis. More precise estimates of population miracidial output through different modes of transmission should follow from other, more extensive studies in different settings.

The importance of hygienic washing becomes intuitively clear from the following calculation. Figure 1 shows that a full anal wash and 2 g of stool result in about the same average number of miracidia for an individual with a given epg. To arrive at the population miracidial output, the anal wash result is multiplied by around 720, i.e. 1000 individuals $\times$ 1.6 times defecating per day $\times$ 0.45 times hygienic washing. The result for 2 g fresh stool is multiplied by 60 to arrive at the average stool weight of about 120 g (note that the reported values of 130 g and 194 g represent 24 h stools). The ratio of

720/60 is exactly the value of 12, which resulted from the more detailed calculations using information from Table 1. Another conclusion that could be drawn from Figure 1 is that the amount of stool adhering to the peri-anal region after defecation is about the same as that of a fresh stool sample, thus 2 g. Differences in viability or concentration of eggs (Yu et al., 1998) may cause slight deviations, however. The exact amount of stool adhering to the peri-anal region, and the incorporated number of viable schistosome eggs, can only be obtained from additional studies.

Husting (1965) was the first to suggest that hygienic washing in streams is a probable route of contamination of water bodies with *S. mansoni* eggs. Also, Chandiwana (1986) reported that eggs remaining around the peri-anal region could be a major factor in the transmission of schistosomiasis mansoni, which would explain the continuation of transmission in the hot dry period in Zimbabwe. Furthermore, Cheesmond and Fenwick (1981) considered the transfer of eggs through bathing and washing of hands an evident way of contamination of water bodies in Egypt. We are the first to have experimentally demonstrated the validity of *S. mansoni* transmission through hygienic bathing.

As surface waters are common goods for drinking and other domestic activities, defecation into streams is not accepted and prohibited in traditional societies such as in Northern Senegal. This was confirmed by regular observational studies on water contact behavior in the same study area throughout a period of 3 years, which never resulted in a single observation of direct defecation into the water, even by children (unpublished data). We are aware that the presence of an observer may have resulted in more desirable behaviors, but even if people occasionally defecate directly into the water, it is very unlikely to have happened to an extent of a dozen of complete stools in the water every day in a population of about 1000 individuals. Fecal material may also reach the water through accidental events, such as animals walking through defecation sites and carrying human feces into the water. However, there is no written evidence of such events, and the amount of fecal material transferred would probably be limited. Moreover, it has been shown that eggs in naturally deposited feces lose their viability within a couple of hours, certainly in a hot and dry climate as is usual in Northern Senegal (Kassim and Gibertson, 1976; Upatham et al., 1976). Furthermore, the role of an animal reservoir in the transmission of *S. mansoni* is usually considered negligible (Adewunmi et al., 1991; Duplantier and Sene, 2000). These observations, in combination with our study results, suggest a major role for hygienic bathing after defecation in the transmission of *S. mansoni*, at least in the absence of rainfall (i.e. >90% of the days in Northern Senegal).

This finding probably applies to many more African populations endemic for schistosomiasis mansoni. It is, however, questionable whether this will contribute to new or better means of control. In Senegal, as in many parts of Africa, washing or bathing after defecation (and also after urination) is an important cultural practice, more than a religious one (Sow et al., 2004). Given this longstanding tradition, the introduction of alternative options would require substantial and possibly insurmountable behavioral changes. For example, latrines, as an alternative to defecation in the field, have rarely shown to be effective in traditional communities (Mertens et al., 1992; Vu Nguyen et al., 2006). Even putting an end to hygienic washing in streams may not yield the expected positive outcome, because remnants of fecal material of infected people may still be able to contaminate the water during playing or swimming, especially when considering that the eggs may survive for a long time in a moist environment such as the peri-anal region. The likelihood of this possibility can easily be tested using the same methodology as presented in the current study. Last but not least, dissuading hygienic bathing as a control measure may even be detrimental, as the advantages of good hygienic practices could outweigh the disadvantages caused by schistosomiasis.

In conclusion, our study suggests that hygienic bathing after defecation is a major factor in the transmission of *S. mansoni*. It remains to be seen whether alternatives to hygienic washing in streams are successful, or even desirable at all.

Authors' contributions: SS, KP, JV, BG and SJdV designed the field studies; SS and KV conducted the field studies with supervision from KP; SS, KV and SJdV analyzed the data; SS and SJdV drafted the article; KP, JV, BG and SJdV revised the article. All authors read and approved the final manuscript. SJdV is guarantor of the paper.

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Conflicts of interest: None declared.

Ethical approval: This study is part of a larger investigation of schistosomiasis epidemiology, transmission and control in Senegal, for which approval was obtained from the ethical committees of the Institute of Tropical Medicine in Antwerp, Belgium and the Ministry of Health in Dakar, Senegal.

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