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Daily emergence of *Schistosoma mansoni* and *S. haematobium* cercariae from naturally infected snails under field conditions

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Abstract

The daily emergence of *Schistosoma mansoni* and *S. haematobium* cercariae was investigated under field conditions. Intermediate host snails of both schistosome species were collected during the rainy season, cold dry season and warm dry season and kept separately in test tubes in habitat water. Shed cercariae were collected from each of the test tubes at two hourly intervals, transferred to Petri dishes and counted. Mice were exposed to these cercariae to establish the identity of the schistosome parasites. Peak shedding for both species was observed at 1100 h during the rainy and warm dry seasons and at 0900 h during the cold dry season. Shedding before 0900 h was found only for *S. haematobium* in the rainy season while shedding after 1700 h occurred only during this season at both species. Shedding observed during 1900 h observation period was in the low category for both species. No shedding was observed during the 2100 h observation period for any of the species and the investigation was discontinued after this period. Only *S. haematobium* ova were found in the exposed mice.

Introduction

The schistosomiasis endemic areas of South Africa are situated mainly in the Northern Province, Mpumalanga and Kwazulu-Natal. Settlements in these areas are characterized by informal housing without proper water reticulation and sanitation facilities. These circumstances give rise to the situation where the local residents are forced to make use of natural water for domestic and recreational purposes, a situation also present in the Mamitwa village in the Northern Province where this study was conducted. Furthermore, the community structure comprises mainly infants, schoolchildren, mothers and grandparents, while fathers are working in the cities.

Human infection with schistosomes depends largely on the density of cercariae at transmission sites. Other factors include length of contact, time of contact, immunological status of host, etc. Emergence patterns are therefore important in determining the time of the day when the risk of infection is highest. Cercariae of *Schistosoma haematobium* are usually released in high numbers during periods of daylight. Jordan & Webbe (1993) found that cercariae of *S. mansoni* and *S. haematobium* were present in natural water habitats mainly during daylight, while Pitchford *et al.* (1969) established both constant daily, as well as seasonal cercarial emergence patterns. Cercarial periodicity data also have practical applications for persons concerned with health education with respect to schistosomiasis. These data may also be of importance in designing sustainable schistosomiasis control programmes. During this study, which was conducted on site in the field, the daily emergence patterns of both *S. haematobium* and *S. mansoni* were studied.

Materials and methods

Snails (*Bulinus globosus* and *Biomphalaria pfeifferi*) were collected before 0700 h during the rainy season (January–April), the cold dry season (May–August) and the warm dry season (September–December). To screen for emergence of cercariae, snails were kept separately in test tubes each containing 10 ml filtered habitat water. A

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cotton plug prevented the snails from leaving the water. The water in each of the test tubes was separately decanted into Petri dishes after a 2-h shedding period. Snails were then rinsed in filtered water from the habitat and transferred to identical clean test tubes also containing 10 ml of filtered habitat water. Counting commenced directly after each 2-h shedding period and was discontinued after the last 2-h period during which no cercariae were observed. The Petri dishes were supplied with a 5 mm square grid index to facilitate counting. Counting was done by means of stereomicroscopes at a 30 × magnification. The numbers of cercariae were counted, and divided into three intensity groups per observation period, namely low (1–10), medium (11–30), and high (31 or more). Air temperature was determined in the middle of each of the 2-h periods. The volume of water in the test tubes was so small that the test tube temperature did not vary from the air temperature by more than 0.5°C. This procedure was conducted in the shade on the bank of the river and was repeated for three consecutive days during each season.

A second investigation was conducted to establish whether or not any cercariae were shed before 0900 h in the morning. This was done by collecting as many snails as possible of each species late in the afternoon, transferring them to a stainless steel mesh cubicle of 8000 cm^3 and placing them in habitat water until 0300 h the next morning. These snails were screened every two hours until 1900 h for presence of shed cercariae as already described.

Identification of the cercariae shed by *Bulinus globosus*, intermediate host of *S. haematobium* and *S. mattheei*, was also seasonally done by collecting the larvae after each of the 2-h periods. This procedure entailed the following: after counting of the cercariae shed by each snail during each period, they were pooled and transferred to 200 ml of filtered habitat water in a container. Depending on the total number of cercariae present per period, a number of *Mastomys coucha* mice were selected for exposure to these cercariae. Before exposure mice were immersed in filtered habitat water for 10 min, a procedure which stimulated them to empty their gut and urinary bladder contents, thereby limiting contamination of the cercariae exposure medium. The mice were then exposed to the pooled cercariae in the container with 200 ml water, for a period of 2 h. The size of the container was such that it allowed free movement of the mice without the risk of drowning. After the exposure period mice were transported to the laboratory and kept for three months after which they were dissected. The liver of each mouse was removed and homogenized and the homogenate passed through a Visser filter® to isolate the ova. On account of the fact that only typical *S. haematobium* ova were recovered, identification of the ova by means of a stereomicroscope only was considered sufficient for this investigation.

Results

The results of this investigation are presented in tables 1–7. From table 1 it is clear that the prevalence of infected snails was respectively 10.2%, 26.5% and 22.4% for the rainy, cold dry and warm dry seasons in the case of *Bulinus globosus*. The corresponding values for *Biomphalaria pfeifferi* were 16.6%, 8.8% and 10.8%.

The results of cercarial shedding by *Bulinus globosus* during the rainy season (table 2) revealed that only low intensity shedding took place before 0700 h and that only 7.1% of the snails were involved in shedding. Low, medium and high shedding patterns were observed from 0900 h until 1500 h. A peak in high intensity shedding was observed for the 0900–1100 h shedding period. Only low cercarial shedding was observed for the 1700–1900 h period.

With regard to cercarial shedding patterns observed during the cold dry season for this intermediate host, it is evident that no cercariae were shed until the 0700–0900 h observation period (table 3). A peak in the high intensity category and shedding in the medium and low intensity category were also observed during this period. No shedding was observed for the 1700–1900 h period. Similar results were found for the warm dry season except that a peak in high intensity shedding was observed for the 0900–1100 h period (table 4). Only low intensity shedding by 13.1% of the snails was observed for the 1500–1700 h period and no shedding was observed thereafter.

Cercarial shedding patterns observed from *Biomphalaria pfeifferi*, intermediate host of *S. mansoni*, during the rainy season showed that no shedding took place before 0900 h (table 5). Only medium intensity shedding was observed for the 0700–0900 h period while a peak in high intensity shedding was observed for the 0900–1100 h period. Only low intensity shedding was evident for the 1500–1700 h and 1700–1900 h periods where 30% and 10% of the snails respectively, were involved. From the results obtained for the cold dry season it is clear that no cercarial shedding was observed before the 0700–0900 h period (table 6). A peak in high intensity shedding was also observed at this stage. All snails that shed cercariae during this period did so in the high intensity group. Only low intensity shedding was observed for the 1500–1700 h period while no shedding was observed thereafter. More or less similar results were found with regard to emergence patterns observed for the warm dry season (table 7). No cercariae were observed before the 0700–0900 h shedding period. Only high intensity shedding was observed during this period while a peak in high

Table 1. The total number of *Bulinus globosus* and *Biomphalaria pfeifferi* snails collected during the rainy, cold dry and warm dry seasons and the number of each species that were infected.

Snail species	Rainy season	Cold dry season	Warm dry season
B. globosus			
Number collected	410	188	169
Number infected	42	50	38
% infected	10.2	26.5	22.4
B. pfeifferi			
Number collected	240	360	332
Number infected	40	32	36
% infected	16.6	8.8	10.8

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Table 2. The percentage of *Bulinus globosus* snails shedding cercariae in the low, medium and high intensity categories during the rainy season.

Observation time (h)	0500	0700	0900	1100	1300	1500	1700	1900	2100
Temperature (°C)	24	27	31.5	31	33	30.3	28.6	26	25.3
Low intensity	7.1	7.1	11.9	57.1	11.9	26.1	26.1	7.1	0
Medium intensity	0	0	19.0	7.1	11.9	7.1	11.9	0	0
High intensity	0	0	7.1	33.3	30.9	26.1	0	0	0
No shedding	92.8	92.8	61.9	2.3	45.2	40.4	61.9	92.8	100

Table 3. The percentage of *Bulinus globosus* snails shedding cercariae in the low, medium and high intensity categories during the cold dry season.

Observation time (h)	0500	0700	0900	1100	1300	1500	1700	1900	2100
Temperature (°C)	15.8	15.8	20.9	24	25.7	25.7	20.9	19.6	17.3
Low intensity	0	0	2	10	42	54	32	0	0
Medium intensity	0	0	8	28	20	16	4	0	0
High intensity	0	0	60	46	26	8	8	0	0
No shedding	100	100	30	16	12	22	56	100	100

Table 4. The percentage of *Bulinus globosus* snails shedding cercariae in the low, medium and high intensity categories during the warm dry season.

Observation time (h)	0500	0700	0900	1100	1300	1500	1700	1900	2100
Temperature (°C)	17.3	19.8	23.1	24.8	26.3	27.3	25.9	21.5	19.3
Low intensity	0	0	21.0	21.0	31.5	78.9	13.1	0	0
Medium intensity	0	0	21.0	10.5	31.5	0	0	0	0
High intensity	0	0	42.1	55.2	21.0	10.5	0	0	0
No shedding	100	100	14.2	13.1	15.7	10.5	86.8	100	100

Table 5. The percentage of *Biomphalaria pfeifferi* snails shedding cercariae in the low, medium and high intensity categories during the rainy season.

Observation time (h)	0500	0700	0900	1100	1300	1500	1700	1900	2100
Temperature (°C)	24	27	31.5	31	33	30.3	28.6	26	25.3
Low intensity	0	0	0	42.5	30	20	30	10	0
Medium intensity	0	0	10	20	20	17.5	0	0	0
High intensity	0	0	0	42.5	17.5	20	0	0	0
No shedding	100	100	90	0	32.5	42.5	70	90	100

Table 6. The percentage of *Biomphalaria pfeifferi* snails shedding cercariae in the low, medium and high intensity categories during the cold dry season.

Observation time (h)	0500	0700	0900	1100	1300	1500	1700	1900	2100
Temperature (°C)	15.8	15.8	20.9	24	25.7	25.7	20.9	19.6	17.3
Low intensity	0	0	0	28.1	0	28.1	28.1	0	0
Medium intensity	0	0	0	0	56.2	34.3	0	0	0
High intensity	0	0	100	62.5	6.3	0	0	0	0
No shedding	100	100	0	9.3	37.5	37.5	71.8	100	100

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Table 7. The percentage of *Biomphalaria pfeifferi* snails shedding cercariae in the low, medium and high intensity categories during the warm dry season.

Observation time (h)	0500	0700	0900	1100	1300	1500	1700	1900	2100
Temperature (°C)	17.3	19.8	23.1	24.8	26.3	27.3	29.5	21.5	19.3
Low intensity	0	0	0	0	0	13.8	58	0	0
Medium intensity	0	0	0	0	13.8	0	0	0	0
High intensity	0	0	36.1	100	86.1	86.1	27.7	0	0
No shedding	100	100	63.8	0	0	0	13.8	100	100

intensity shedding occurred during the 0900–1100 h period. No shedding was observed after 1700 h.

The results of the investigation conducted to identify the parasites shed by *Bulinus globosus* revealed that all cercariae shed by this snail species probably belonged to *S. haematobium* because no ova of *S. mattheei* could be found in any of the exposed mice.

Discussion

A similarity in the occurrence of peaks in high intensity shedding during the three seasons investigated for the two parasite species was noticeable in this investigation. During the rainy season, this peak was evident for both species for the 0900–1100 h period, during the cold dry season for the 0700–0900 h period and during the warm dry season for the 0900–1100 h period. Shedding before 0900 h was only found for *S. haematobium* in the rainy season when cercariae were already observed during the 0300–0500 h period. Only 7.1% of snails were involved in shedding during this period and the 0500–0700 h period and shedding was only in the low intensity category. No *S. haematobium* or *S. mansoni* cercariae were shed before 0900 h during any of the remaining seasons. It is further evident that cercarial shedding of both species after 1700 h was observed only during the rainy season. The shedding observed during the 1700–1900 h period for both species was only in the low intensity category and only 7.1% and 10% of *Bulinus globosus* and *Biomphalaria pfeifferi* were respectively involved. No cercariae of any of the species were observed for the 1900–2100 h period and the investigation was discontinued after this period. Nojima & Sato (1978) showed peak production of cercariae of *S. haematobium* at 1300 h following four hours of continuous illumination while Raymond & Probert (1987) showed a large peak at 1100 h which compares well with the current study. The peak at 2000 h for *S. haematobium* found by these authors was not present during this study. It is, however, possible that there could have been a peak after the 2100 h period that was not observed because the investigation was already discontinued at that time. According to Rowan (1958) the release of cercariae from snails in the field apparently occurs primarily from 0900 h to 1600 h with the most rapid emergence at noon. This conclusion supports the laboratory findings of Faust & Hoffman (1934) and Giovannola (1936) who reported that cercariae emerge from 0900 h to 1400 h, particularly when the snails are exposed to sunlight. Giovannola (1936) noted that emission of cercariae is highest from 0930 h to 1500 h. Pitchford *et al.* (1969) found that most of the cercariae of *S. mansoni* and *S. haematobium* were shed between 1100 h and 1400 h during the summer. In a study by Tchuente *et al.* (1999) on *B. pfeifferi* from Ndombo, near Richard-Toll, Senegal, a peak in cercarial shedding around mid-day was found. In a more recent study by Southgate *et al.* (2000) on *B. pfeifferi* from Ndiangue, Senegal, infected with *S. mansoni* from Nkolbisson from Cameroon, revealed a circadian rhythm with one shedding peak at mid-day. These results correspond favourably with the current investigation for all the seasons investigated.

Cercarial periodicity data have practical applications for persons concerned with health education of the public in the schistosomiasis endemic areas in South Africa. During this investigation the authors have noticed that members of most of the families, because of a lack of a functioning water reticulation system, consistently draw their water for drinking, or do their washing and bathing, at the transmission sites also during hours which coincide with the peak of cercarial abundance. Slight changes in the time of water usage might reduce exposure to cercariae considerably. Although viability and infectivity of the cercariae should be taken into account in determining safe periods for water contact, their impact would depend on specific habitat condition, such as turbidity, water velocity, habitat size and depth, etc. By taking these factors into account and with regard to the actual time of peak shedding observed during this study, the safest period for use of water would appear to be from 0500 h to 0900 h or after 1900 h.

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