

RESEARCH NOTE

Cercarial Emergence of *Schistosoma mansoni* from *Biomphalaria glabrata* and *Biomphalaria straminea*

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Biomphalaria glabrata is the most susceptible intermediate host of *Schistosoma mansoni* in Brazil; *Biomphalaria straminea*, in contrast, shows the highest resistance to infection. However, *B. straminea* is an important vector of schistosomiasis in Northeast Brazil due to its widespread distribution and association with high prevalence of human schistosomiasis in endemic areas (WL Paraense, LR Corrêa 1989 *Mem Inst Oswaldo Cruz* 84: 281-288).

Temporal aspects involved in the passing of *S. mansoni* cercariae from the snail to the definitive host are relevant for understanding the dynamics of schistosomiasis transmission because the parasite can only complete its life cycle if a spatiotemporal coincidence occurs between the cercariae and the definitive host. Nevertheless, studies of snail/parasite relationships regarding temporal aspects of cercarial emergence are carried out mainly on the *B. glabrata*/*S. mansoni* combination.

In the present work, the emergence of *S. mansoni* cercariae from *B. glabrata* and *B. straminea* was compared through the following parameters: duration of the prepatent period, proportion of snails shedding cercariae, daily av-

erage of cercarial output, duration of patent period, and peak hours (acrophase) of cercarial emergence.

A total of 430 *B. glabrata* snails from Belo Horizonte, MG (BH2 stock), 3-8 mm in shell diameter and 1,145 *B. straminea* snails from Picos, PI (Picos stock), 3-8 mm in shell diameter, were exposed individually to five *S. mansoni* miracidia isolated from the same biotope as their snail hosts. The *S. mansoni* from Belo Horizonte (BH2 strain) was isolated in 1985 from naturally infected snails (Paraense, Corrêa *loc. cit.*), whereas the *S. mansoni* from Picos was isolated by WL Paraense in 1980 from faeces of an autochthonous patient (EC strain). Both snail stocks have been kept in the Department of Malacology, Oswaldo Cruz Institute, Rio de Janeiro. The two *S. mansoni* strains have been kept by passages through syntopic *B. glabrata* (BH2) and *B. straminea* (Picos), and female Swiss albino mice. Once a year, the EC strain was passed through BH2 *B. glabrata* to avoid losing the parasite.

The techniques for obtaining miracidia and infecting snails were as described by Paraense and Corrêa (*loc. cit.*). After being exposed to the miracidia, the snails were kept in the laboratory in 5-liter glass containers with dechlorinated tap water (up to 10 snails per litre) at 25-29°C. Fresh lettuce was provided as food source in excess of requirements. Water and food were renewed at least once a week. Screenings for positive snails started on the 25th day after exposure to miracidia and was repeated three times a week for two months thereafter. For screening, the snails were isolated in vials with water and exposed up to 1 hr to the light of electric lamps (30 ± 1°C) to induce cercarial emergence. This procedure permitted estimation of the duration of the prepatent period as the time (in days) elapsed from exposure to miracidia to the first record of cercarial emergence. The positive snails were transferred to an outdoor area under natural conditions of temperature (26.7 ± 3.3°C), light phase (12 ± 1 hr) and light intensity (from 88 to 2800 lux). Each snail was kept isolated in a numbered glass container in 200 ml of dechlorinated water and fresh lettuce which were both renewed twice a week. This isolation permitted determination of the parasitological and chronobiological parameters based on data from each snail. After the last screening, the proportion of snails shedding cercariae was calculated in relation to the number of snails exposed to miracidia. A sample (40%) of *B. straminea* snail that survived more than 90 days without shedding cercariae was dissected under a stereomicroscope and examined for the presence of sporocysts in the body tissues.

The emergence of cercariae was followed in a sample of at least 40% of the positive snails until their death. This procedure permitted estimation of the daily average cercarial output and the duration of patent period as well as detecting the presence of any circadian rhythm in each snail. For quantifying the cercarial output, the positive snails were placed separately in acrylic vials with 4 ml of dechlorinated water and a 1 cm disc of fresh lettuce for 24 hr once a week. The snails were transferred to new vials and the residual was filtered and the cercariae counted (Paraense, Corrêa *loc. cit.*). For detecting circadian rhythms cercaria counts were carried out at 3-hr intervals over three days to give total cercarial output and to estimate the proportion of cercariae emerging in the diurnal (6:00-18:00 hr) and nocturnal (18:00-6:00 hr) phases. Both the quantification of cercarial output and the detection of circadian rhythm were carried out in the outdoor area from November to December.

The χ^2 test was applied to compare the proportion of snails shedding cercariae between the two snail species. The Mann-Whitney test was used for comparing statistically the duration of prepatent period, daily average cercarial output, and duration of patent period between *B. glabrata* and *B. straminea*. For chronobiological analysis, the data were examined in a time series, i.e. the number of emerging cercariae at each interval for three consecutive days plotted against time. The data were transformed mathematically $-\log(x+1)$ in order to satisfy requirements of the Single Cosinor Analysis (F Halberg et al. 1977 *Chronobiologia* 49: 1-190) fitting a 24-hr cosine curve with a 5% significance level. The acrophases were estimated for each snail and compared through the limits of the 95% confidence intervals. They were considered significantly different among the snails whenever their confidence intervals did not overlap (W Nelson et al. 1979 *Chronobiologia* 6: 305-323).

Judged by detection of cercarial shedding, 14.9% (64/430) *B. glabrata* became infected significantly more than the 0.5% (5/1,145) *B. straminea* ($\chi^2 = 138.8$; $p < 0.001$). No sporocysts were detected by dissection in the tissues of 344/860 *B. straminea* still surviving 90 days after exposure. Twenty nine snails of the former species and four of the latter were followed until their death (Table). The daily average cercarial output was significantly higher in *B. glabrata* than in *B. straminea* ($p < 0.05$), but the lengths of the prepatent and patent periods did not differ significantly between species. As these analyses are based on a small sample of *B. straminea*, further studies are necessary to confirm them.

It is interesting to note that the cercarial out-

put obtained for *B. glabrata* in the present study is substantially less than that commonly found in laboratory studies (RF Sturrock 1993 *The intermediate hosts and host-parasite relationships* p. 33-85 In *Human Schistosomiasis*, Cab International, Wallingford, UK). If the present data on 29 *B. glabrata* snails are extrapolated to estimate the total cercarial output from 64 out of 430 exposed snails, and then used to calculate the TPC/100 index (F Frandsen 1979 *Z Parasitenkd* 58: 275-296), the result ($94,702.70 \times 64/29 \times 100/430 = 48,604.42$) indicates a poorly compatible combination (Class II). This apparently low level of cercarial production may be due to the use of natural conditions of light and temperature during the period of cercarial emergence. As laboratory observations are usually based on forced cercarial shedding (Sturrock *loc. cit.*), it is not surprising that the cercarial outputs obtained under such artificial conditions were much higher than that in the present work. Future studies aiming at quantifying cercarial output should take into account possible differences between spontaneous and induced emergence of cercariae.

Cercarial emergence was mainly diurnal both in *B. glabrata* (98.5%) and *B. straminea* (95.5%). Circadian rhythms were detected among 76% (22 out of 29) of *B. glabrata* and in three of the four *B. straminea* snails. The acrophases occurred between 11:58-14:45 hr in *B. glabrata* and 13:57-15:59 hr in *B. straminea*, with confidence intervals varying from 9:33-19:19 hr and 12:55-17:42 hr, respectively (Table). These results suggest the existence of a circadian rhythm of cercarial emergence in *B. straminea* and confirm those of other authors for *B. glabrata* (A Théron 1985 *Vie Milieu* 35: 23-31). The chronobiological analysis indicated that the acrophases of cercarial emergence were similar within each snail species, thus suggesting that, despite variability among the snails, peak cercarial emergence tends to occur in particular hours of the day. This is in accord with the findings of other authors working with different *B. glabrata*/*S. mansoni* combinations under varying experimental conditions, either using the chronobiological (JL Chassé, A Théron 1988 *Chronobiol Int* 5:433-440) or other approaches (WB Rowan 1958 *Ann Trop Med Hyg* 7: 374-381).

The two snail species showed similarities in the daily peaks of cercarial emergence, as indicated by an overlap in all confidence intervals of the acrophases. Thus, the acrophases were between 13:00 hr and 16:00 hr in 17 out of 22 (77.3%) of *B. glabrata* snails as well as in the three *B. straminea* snails that showed a circadian rhythm in cercarial emergence. This finding is relevant for understanding schistosomiasis transmission in

TABLE

Cercarial output and circadian rhythm of cercarial emergence in individual snails of *Biomphalaria straminea* (Picos, Brazil) and *B. glabrata* (Belo Horizonte, MG, Brazil) infected with the syntopic strain of *Schistosoma mansoni*. Duration of prepatent and patent periods are also given

Snail number	Prepatent period (days)	Patent period (days)	Cercarial output			Circadian rhythm		
			Samples examined	Total count	Daily average	Presence	Acrophase (hr:min)	95% confidence intervals (hr:min)
<i>B. straminea</i>								
1	32	63	9	987	109.7	yes	13:57	12:55 - 15:00
2	30	11	1	57	57.0	yes	15:29	14:19 - 16:38
3	23	11	1	11	11.0	no	—	—
4	29	13	2	34	17.0	yes	15:25	13:08 - 17:42
<i>B. glabrata</i>								
1	29	15	2	104	52.0	yes	13:51	11:11 - 16:32
2	29	23	3	356	118.7	yes	13:53	11:14 - 16:31
3	29	54	5	412	82.4	yes	14:55	13:17 - 16:32
4	29	21	3	416	138.7	yes	13:11	10:54 - 15:29
5	29	33	4	224	56.0	yes	15:07	13:19 - 16:54
6	29	41	5	271	54.2	yes	14:26	12:20 - 16:32
7	29	33	4	171	42.8	yes	14:10	12:03 - 16:18
8	29	54	5	767	153.4	yes	13:40	11:31 - 15:49
9	29	8	1	73	73.0	yes	16:01	13:41 - 18:21
10	29	29	3	361	120.3	no	—	—
11	29	46	5	414	82.8	yes	14:24	12:23 - 16:25
12	29	9	1	102	102.0	yes	12:39	10:12 - 15:07
13	28	21	3	48	16.0	no	—	—
14	28	29	3	247	82.3	yes	12:49	10:14 - 15:24
15	28	33	4	359	89.8	yes	14:10	12:08 - 16:11
16	28	15	2	91	45.5	yes	14:22	12:32 - 16:07
17	28	22	3	382	127.3	yes	15:36	12:49 - 18:23
18	28	15	2	288	144.0	yes	13:39	11:27 - 15:50
19	28	29	3	458	152.7	no	—	—
20	28	36	4	658	164.5	yes	11:58	09:33 - 14:23
21	28	37	4	215	53.8	yes	15:19	12:37 - 18:00
22	28	23	3	56	18.7	yes	16:45	14:11 - 19:19
23	28	22	3	59	19.7	yes	14:23	12:09 - 16:38
24	28	33	4	379	94.8	no	—	—
25	28	82	7	1,640	234.3	yes	13:27	11:30 - 15:24
26	28	21	3	671	223.7	yes	13:36	11:56 - 15:17
27	35	76	7	532	76.0	no	—	—
28	34	23	3	27	9.0	no	—	—
29	34	23	3	484	161.3	no	—	—

the areas concerned, as it suggests that the afternoon is the part of the day with highest risk of infection. Local studies on the association between the temporal aspects of cercarial emergence and water-contact patterns of human hosts are needed to confirm this hypothesis.

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