RESEARCH NOTE

Longitudinal Study of Circadian Rhythms in the Cercarial Emergence of *Schistosoma mansoni* from *Biomphalaria glabrata*

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Key words: *Schistosoma mansoni* - longitudinal circadian rhythms - *Biomphalaria glabrata* - cercarial emergence

Longitudinal studies of *Biomphalaria glabrata* infected with *Schistosoma mansoni* are relevant to a better understanding of the dynamics of schistosomosis transmission. They permit a more detailed evaluation of the compatibility between the parasite and intermediate host, as well as the detection of cercarial emergence patterns over time (WHO, Technical Report Series 830, 1993, 85pp.). It is known that *S. mansoni* cercarial emergence can last aproximately a year in *B. glabrata* (MA Stirewalt 1954 *Exp Parasitol 33*: 504-516), and that it occurs mainly in the diurnal phase (CE Faust, WA Hoffman 1934 *J Publ Hlth Trop Med 10*: 1-97) according to a circadian rhythm whose peak often correlates with periods of water contact activities of the vertebrate host (A Théron 1985 *Vie et Milieu 35*: 23-31, 1985 *Ann Parasitol Hum Comp 60*: 665-674, 1986 *Parasitol Today 2*: 192-194). However, no longitudinal study has evaluated circadian rhythms of emergence throughout the course of cercarial period of individual snails.

The present study investigated circadian rhythms in the emergence of *S. mansoni* cercariae from individual *B. glabrata* snails during the patent period. For this purpose, the acrophase (peak hour) of cercarial emergence was estimated for each snail by the Single Cosinor Analysis (F Halberg et al. 1977 *Chronobiologia* (Suppl I) *49*: 1-190). The duration of prepatent (F Frandsen 1979 *Z Parasitenkd 58*: 275-296) and patent periods as well as the cercarial output were also individually recorded.

A total of 398 specimens of *B. glabrata* from Belo Horizonte, Brazil, 4-5mm in shell diameter, were exposed individually to five *S. mansoni* miracidia from a sample isolated from the same biotope as their snail hosts. The parasite was isolated in 1985 from naturally infected snails (WL Paraense, LR Corrêa 1989 *Mem Inst Oswaldo Cruz 84*: 281-286). Both the snail and parasite were provided by the Department of Malacology of Oswaldo Cruz Institute, Rio de Janeiro. The miracidia were obtained from eggs concentrated from feces of infected mice (5th passage) by sedimentation (A Lutz 1919 *Mem Inst Oswaldo Cruz 11*: 109-140). After exposure to miracidia, groups of up to forty snails were kept in 4 l glass containers with dechlorinated tap water at 25 ± 2°C. The snails were fed every two days with fresh lettuce *ad libitum*; water was renewed weekly. Screening to detect the positive snails started on the 21st day after exposure to miracidia and was repeated three times a week. For screening, the snails were exposed for 2 hr to the light of electric lamps (60 W) to stimulate cercarial emergence. The positive snails were transferred to an outdoor area at least a week before the chronobiological tests and kept individually in 160 ml glass containers with dechlorinated tap water and fresh lettuce *ad libitum*. The tests were carried out two to three times a month under natural conditions of temperature (varying from 19° to 34°C) and light (light phase of 12 ± 1hr and intensity varying from 0.17 to 11,000 lux). Each test consisted of quantification of cercarial emergence at 3hr intervals for two consecutive days. During these days the snails were kept individually in acrylic vials with 4 ml of dechlorinated water and fed with fresh lettuce. Every 3hr the snails were transferred to new vials. The remaining suspension was then filtered (Paraense, Corrêa *loc. cit.*) and the filtered cercariae were counted exhaustively under a stereomicroscope.

Cercarial counts from individual snails were used to calculate the percentages of daytime (from 6:00 to 18:00) emergence. They were evaluated chronobiologically through the Single Cosinor Analysis used for detecting 24 hr rhythms and estimating the acrophases of cercarial emergence for snails which had been tested at least three times. The acrophases were compared pairwisely and considered significantly different whenever their 95% confidence intervals did not overlap (W Nelson et al. 1979 *Chronobiologia 6*: 305-323).

This work received financial support by FAPERJ.
Received 5 October 1994
Accepted 29 March 1995

TABLE

Cercarial emergence of *Schistosoma mansoni* from individual *Biomphalaria glabrata* snails (BH combination) by the weeks of infection. The percentage of day time emergence of cercariae, as well as the results of the chronobiological tests to detect circadian rhytms through the Single Cosinor method are presented. The acrophases (h:min) and respective 95% confidence intervals are given whenever circadian rhythms were detected. The total number or cercariae emerged per snail during the 48 hr of each test are also shown. The duration of prepatent and patent periods for each snail are also given

Snail	Duration (days)		Parameters of Cercarial output and Circadian Rhythm	Times of Chronobiological tests (weeks of cercarial period)						
	Prepatent period	Patent period		0	2	4	7-8	9-10	12-13	14
1	28	83	Total cercariae	81	224	100	+			
			% diurnal cercariae	98.8	91.9	90.0				
			Acrophases	14:12	15:08	NS				
			Confidence intervals	12:35-15:48	12:55-17:21					
2	33	58	Total cercariae	33	391	267	+			
			% diurnal cercariae	66.7	93.4	69.3				
			Acrophases	NS	15:24	NS				
			Confidence intervals		13:24-17:24					
3	33	58	Total cercariae	121	286	644	+			
			% diurnal cercariae	99.2	51.4	85.7				
			Acrophases	14:41	16:11	15:45				
			Confidence intervals	12:35-16:47	14:30-17:51	13:35-17:54				
4	26	108	Total cercariae	501	2,467	265	319	116	848	+
			% diurnal cercariae	95.4	99.9	94.0	92.2	100	100	
			Acrophases	15:49	13:37	12:13	14:32	14:48	14:13	
			Confidence intervals	15:29-17:09	12:29-14:45	11:09-13:18	12:32-16:32	12:36-17:00	12:40-15:45	
5	23	64	Total cercariae	329	443	281	5	+		
			% diurnal cercariae	74.8	100	88.3	60.0			
			Acrophases	18:03	15:21	15:33	*			
			Confidence intervals	17:00-19:06	13:11-17:31	13:39-17:26				
6	23	39	Total cercariae	1,010	52	21	+			
			% diurnal cercariae	97.7	100	100				
			Acrophases	15:51	14:41	*				
			Confidence intervals	14:33-17:08	12:37-16:44					

(+) snail found dead; (*) number of cercariae not enough for the analysis; (NS) non-significative rhythm

The following parameters were also recorded for each snail: (a) duration of prepatent period (days): since exposure to miracidia until the first cercarial emergence. (b) duration of patent period (days): between the first and last cercarial emergence, and (c) cercarial output: total number of cercariae emerged per snail in each test.

Only six out of 62 infected snails survived long enough for at least three tests while shedding cercariae; no self-cure was observed (Table). The first cercarial emergence occurred between the fourth and the sixth weeks after exposure with mean and standard deviation of 27.7 ± 4.5 days. The duration of the patent period (62.3 ± 24.0 days) varied from 39 to 108 days. Cercarial output per snail per test (48 hr) varied from 5 to 2,467. Of the 8,804 cercariae that emerged from all snails in all tests, 11.4% emerged at nighttime. Circadian rhythms in cercarial emergence were detected in all six snails, of which two snails (3 and 4) showed a circadian rhythm in every test. The four remaining snails showed this rhythm in at least one test. The acrophase estimates of cercarial emergence varied from 12:13 to 18:03 with 76.5% of them occurring between 14:00 and 16:00 pm. The only acrophase shown to be significantly different among snails was that of snail 5 in week 0. The acrophases estimated for each snail did not differ significantly within the tests, with the exception of snail 4. In this case, the acrophase corresponding to week 0 was significantly later than those of weeks 2 and 4.

Various authors have observed a great interindividual variability in the cercarial output of *S. mansoni* from *B. glabrata* snails (FS Barbosa et al. 1954 *Publ Avul CPqAM 7*: 79-92, FS McClelland 1965 *Bull WHO 33*: 270-276, CP Souza et al. 1983 *Mem Inst Oswaldo Cruz 78*: 251-256). In the present study, a substantial intraindividual variability in cercarial output was also detected, confirming earlier results for this species (FG Schreiber, M Schubert 1949 *J Parasitol 35*: 91-100). In addition, cercarial emergence at nighttime was not negligible. Thus, for a proper determination of daily cercarial output in a given *S. mansoni/B. glabrata* combination, cercarial counts should be obtained at different times along the cercarial period, including nocturnal phases.

Cercarial emergence clearly showed a circadian rhythm. However, the present study revealed that this pattern is intermittent. Therefore, it is suggested that, if possible, chronobiological tests on cercarial emergence should be performed at monthly intervals throughout the course of the patent period.

The peak hours of cercarial emergence showed relatively little inter- and intra-individual differences during the patent period, tending to occur in mid-afternoon. This finding is epidemiologically relevant as it shows which particular hours of the day are the most dangerous for infection of vertebrate hosts. This implies in an accumulation of cercariae and a high and prolonged risk of infection in the afternoon and early evening in transmission sites with no flow. In a flowing stream, the cercariae would be rapidly swept away and the danger period might be relatively short (A Théron 1982, p. 289-292 In DF Mettrick, SS Desser (eds) *Parasites - Their world and Ours* Elsevier Biomedical Press).

Acknowledgements: to Dr Ligia Côrrea and Dr Wladimir Lobato Paraense, Departamento de Malacologia, Instituto Oswaldo Cruz, for the training facilities, as well as for providing the snail and parasite stocks. To Ana Amélia Benedito Silva, Grupo Multidisciplinar de Desenvolvimento e Ritmos Biológicos, Universidade de São Paulo, for help with the chronobiological analysis of the data.