EMERGENCE OF *OPISTHORCHIS VIVERRINI* CERCARIAE FROM NATURALLY INFECTED *BITHYNIA* (*DIGONIOSTOMA*) *SIAMENSIS GONIOMPHALOS*

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Abstract. Under natural conditions, the emergence of *Opisthorchis viverrini* cercariae from naturally infected *Bithynia* (*Digoniostoma*) *siamensis goniomphalos* showed diurnal periodicity, peaking between 8:00-10:00 AM. The cercariae did not emerge during darkness, but low-intensity light could induce a release. Cercariae shedded from each field infected *B.*(*D.*) *s. goniomphalos* was recorded daily. The maximum output from one snail was 1,728 cercariae in a day. The total cercarial output from all five infected snails was 56,555 and the maximum of total cercariae shed from one snail was 27,692. The field-infected *B.* (*D.*) *s. goniomphalos* could survive for 70 days after the snails were collected.

INTRODUCTION

The emergence of cercariae from their snail hosts requires many ecological and physiological factors. Many studies have been done on the blood fluke, *Schistosoma*. The patterns of cercarial emergence were not consistent. Mao *et al* (1949) found that light played an important role in the emergence of *Schistosoma japonicum* cercariae from their Chinese snail host, *Oncomelania hupensis*, while Bauman *et al* (1948) found that the natural release of *S. japonicum* cercariae from *Oncomelania quadrasi* was noctural. Asch (1972) studied rhythmic emergence of *S. mansoni* cercariae from *Biomphalaria glabrata* under illumination and found that light induced cercarial emergence. Nojima and Sato (1978) also found diurnally periodic emergence of *S. mansoni* and *S. haematobium* cercariae from *Biomphalaria pfeifferi* and *Bulinus globosus,* respectively. Lohachit *et al* (1980) noted that a liberation of *S. mekongi* cercariae from *Neotricula aperta* (β-race) occurred in daytime.

The emergence of liver fluke cercariae from bithyniid snails has not yet been studied. This study conducted laboratory tests to investigate: 1) the pattern of emergence of cercariae of *Opisthorchis viverrini* (Wykoff *et al*, 1965) from field-infected *B.* (*D.*) *s. goniomphalos,* and, 2) the total number of cercariae released from each field-infected *B.* (*D.*) *s. goniomphalos*, under natural conditions.

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MATERIALS AND METHODS

The study used five naturally infected *B.* (*D.*) *s. goniomphalos* collected from Kalasin Province, northeast Thailand, on 18 December 2003. The snails were subsequently induced to estivate, to dry at room temperature, and were wrapped in newspaper for transportation to the laboratory at the Faculty of Tropical Medicine, Mahidol University, Bangkok. The snails arrived at the laboratory on 25 December 2003 and were reared and fed with diatoms in 2.0 × 9.0 cm. Petri dish culture until the experiments started. The snails had an average size of 6.0 × 10.0 mm.

In the first study, the emergence pattern of *O. viverrini* from *B.* (*D.*) *s. goniomphalos* was done on 30-31 December 2003. Each infected *B.* (*D.*) *s. goniomphalos* was put in a 5.0 × 1.5 cm Petri dish, half-filled with conditioned tap water. The snails were left near a window throughout the experiment, to expose the cercariae recorded. Each snail was cleaned with tissue paper before transfer to a new 5.0 × 1.5 cm Petri dish that changed at every 2-hour interval, from 6:00 AM to 4:00 PM of the next day. Room and water temperatures, and intensity of light (Lux meter DIGICOM LX-150), were recorded every two hours during the experiment.

The subsequent study started on 1 January 2004, following the first study, and using the same snails. Each snail was put in a 5.0 × 1.5 cm diatom culture dish half-filled with conditioned tap water. All snails were left in a shaded area near a window to expose them to natural light for the shedding of cercariae throughout the experiment. Each Petri dish culture was changed daily at 8:00 AM and the cercariae from each snail were counted and recorded. Room and water

temperatures, and intensity of light (Lux meter DIGICOM LX-150) were also recorded daily.

RESULTS

Pattern of emergence

The pattern of emergence of *O. viverrini* cercariae from infected *B.* (*D.*) *s. goniomphalos* under natural conditions showed clear diurnal periodicity. The snails started shedding cercariae between 6:00-8:00 AM through 8:00 PM after which the emergences were strictly suppressed until 6:00 AM The peak emergence was between 8:00-10:00 AM. The same pattern was observed on the next day (Fig 1; Table 1).

Cercarial outputs

The total *O. viverrini* cercarial outputs from each naturally infected *B.* (*D.*) *s. goniomphalos* are shown in Table 2. In a day, the lowest cercarial output was zero, while the maximum was 1,728. The cercarial outputs each day varied from time to time. The mean room and water temperatures were 25ºC and 24ºC,

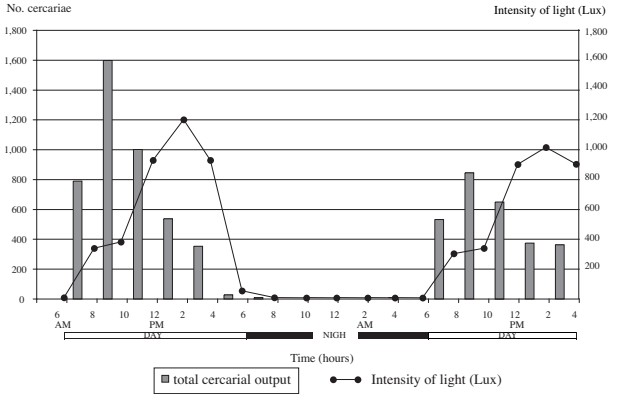

Fig 1- The pattern of two-hour-interval output of *O. viverrini* cercariae from naturally infected *B.* (*D.*) *s. goniomphalos*.

Table 1
The emergence of *O. viverrini* cercariae from field-infected *B.* (*D.*) *s. goniomphalos.*

Time (hours)	Temperature (º C)		Intensity of light (Lux)	Numbers of cercariae emitted by snails nos.					
	Room	Water		1	2	3	4	5	Total
0600-0800	25.0-25.0	24.0-24.0	0-0345	93	32	605	53	7	790
0800-1000	25.0-25.0	24.0-24.0	0345-0380	243	187	631	537	0	1,598
1000-1200	25.0-26.0	24.0-24.5	0380-0900	134	240	173	324	131	1,002
1200-1400	26.0-27.5	24.5-25.5	0900-1200	80	90	23	154	190	537
1400-1600	27.5-27.0	25.5-26.0	1200-0900	39	138	72	0	105	354
1600-1800	27.0-27.0	26.0-26.0	0900-0048	5	2	0	0	20	27
1800-2000	27.0-25.5	26.0-26.0	0048-0	2	0	0	0	7	9
2000-2200	25.5-25.0	26.0-25.0	0-0	0	0	0	0	0	0
2200-2400	25.0-24.0	25.0-24.0	0-0	0	0	0	0	0	0
2400-0200	24.0-24.0	24.0-24.0	0-0	0	0	0	0	0	0
0200-0400	24.0-25.0	24.0-24.0	0-0	0	0	0	0	0	0
0400-0600	25.0-25.0	24.0-24.0	0-0	0	0	0	0	0	0
0600-0800	25.0-25.0	24.0-24.0	0-0320	0	117	220	110	86	533
0800-1000	25.0-25.0	24.0-24.0	0320-0350	0	107	502	115	122	846
1000-1200	25.0-26.0	24.0-25.0	0350-0890	0	95	504	1	50	650
1200-1400	26.0-25.0	25.0-24.0	0890-1000	7	116	248	0	3	374
1400-1600	25.0-26.0	24.0-25.0	1000-0890	195	51	92	0	25	363

Table 2
Total *O. viverrini* cercarial outputs of each naturally infected *B.* (*D.*) *s. goniomphalos.*

Snail no.	Mean no. of cercariae released in a day	Total cercariae released	No. of shedding days
1	117 (0 - 691)	2,575	22
2	334 (0 - 1,385)	15,725	47
3	312 (5 - 1,728)	8,427	27
4	453 (0 -1,433)	27,692	61
5	92 (0 - 532)	2,136	23
	Total	56,555	

respectively. The intensities of light in the morning and afternoon were 0815-1471 Lux. The total cercarial output from all five infected *B.* (*D.*) *s. goniomphalos* was 56,555. The maximum cercarial output from one snail was 27,692.

DISCUSSION

The emergence of *O. viverrini* cercariae from *B.* (*D.*) *s. goniomphalos* under natural conditions showed diurnal periodicity, with a peak between 8:00-10:00 A.M. Like the emergence of cercariae of some blood flukes studied by many authors (Asch, 1972; Nojima *et al*, 1978; Lohachit *et al*, 1980; Mao *et al*, 1980), the cercariae preferred to shed under light conditions, or with diurnal periodicity. We had not designed to study the effect of light on cercarial release; however, the result indicated that *O. viverrini* cercariae emerged from *B.* (*D.*) *s. goniomphalos* at the onset of daylight. Although the peak did not start at the onset of daylight, the cercariae released during the daytime, between 8:00 AM -6:00 PM. A very low light intensity (48 Lux) was sufficient to induce cercariae shedding. Lohachit (2001) noted that infected *B.* (*D.*) *s. goniomphalos* was able to estivate in nature or in the laboratory for at least 25 days, and the maximum number of *O. viverrini* cercarial shed from an infected *B. (D.) s. goniomphalos* after 15 days' estivation was 2,850, and the snail could survive up to 62 days. In our study, the five naturally infected snails were induced to estivate for 7 days; they could emit large number of cercariae (56,555) and could survive for 70 days. The maximum number of cercariae shed from one snail was 27,692.

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