

THE POPULATION DYNAMICS OF CERCARIAE OF *SCHISTOSOMA JAPONICUM* IN *ONCOMELANIA HUPENSIS*

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Abstract. The population dynamics and production of cercariae of *Schistosoma japonicum* in *Oncomelania hupensis* are reported. The experiments covered the whole life span of positive snails and different intervals of cercariae shedding. The results indicated that two patterns of the dynamics of cercariae shedding had been found in the life span of positive snails. The first was a long-time interval (4-7 days) and progressive decline pattern. The cercariae shedding of positive snails lasted 18-19 weeks in males and for 32-33 weeks (once a week). The second was a short-time interval (1-3 days) and continued release pattern. The cercariae shedding of positive snails lasted for 20-36 days (every day shedding). Shedding cercariae stimulate cercariae development.

INTRODUCTION

In close relation to susceptibility, the population dynamics of cercariae of schistosome has been of interest to researchers. From studies on human *Schistosoma* species such as *S. mansoni*, *S. haematobium*, *S. intercalatum* and *S. japonicum*, the results demonstrated that cercariae emergence and production were influenced by light, temperature and the pH value of water (Bauman *et al*, 1948; Mao *et al*, 1949; Komiya and Ishii 1954; Gumble *et al*, 1957; Nojima *et al*, 1980; Williams *et al*, 1984; Kawashima *et al*, 1985), and by different populations of snail hosts and different strains of *Schistosoma* parasites as well (Pages, 1990; Theron, 1986; Fryer *et al*, 1988). Recent studies emphasized more on periodic production and long-term observation (Theron, 1981; Pages *et al*, 1990; Vera *et al*, 1992). The population dynamics of cercariae have a direct effect on parasite transmission. The results may be important for quantitative analysis of epidemiological factors in endemic areas of schistosomiasis.

In this study, the dynamics of cercariae emergence and production of a Chinese strain of *Schistosoma japonicum* in *Oncomelania hupensis hupensis* are reported. The experiments covered the whole life span of positive snails and different intervals of cercariae shedding.

MATERIALS AND METHODS

General methods: Schistosome eggs were obtained from livers of rabbits, which were infected 800-1,000 cercariae of a Chinese strain of *S. japonicum* by using the percutaneous coverslip method and were killed after 45 days infection. Each group was exposed individually to ten miracidia in 12.5 ml culture well for three hours under a strong light. The experimental snails were reared in snail room of temperature 25°C, feeding with mud. After six-weeks infection, the snails shedded cercariae and the positive snails were identified for further observation.

Cercariae were harvested once a week or at different intervals individually. The size of cercariae production lasted 3 hours (09.00-12.00) under a strong light. A few drops of iodine solution were added to cercariae suspension, which was then transferred onto filter paper, where cercariae were counted with aid of a stereomicroscope.

Experiment 1: Two isolates of *S. japonicum* from China were employed in this experiment: isolate 1 from Zhejiang Province, laboratory reared for more than ten years (about 15 generations); isolate 2 from Anhui Province, from field collection. The population of *O. hupensis* was from Zhejiang Province, China. 100 schistosome-free snails (50

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males and 50 females) were exposed individually to 10 miracidia in each group. The dynamics of cercariae shedding were observed once a week and lasted 34 weeks. Every positive snail was separated individually and marked with a number, so that the cercariae production of each snail would be known.

Experiment 2: To assess the cercariae shedding under natural conditions and dynamics of cercariae production during different intervals, this experiment was designed as follows: **Group 1:** every day shedding; **Group 2.** every second day shedding; **Group 3.** every third day shedding; **Group 4.** every fourth day shedding; **Group 5.** every fifth day shedding; **Group 6.** every sixth day shedding; **Group 7.** every seventh day shedding.

After first shedding 10-12 positive snails were divided randomly into one group. The positive snails were kept in small bottles individually and marked the number. The cercariae production was counted per snail per shedding. The dynamics of cercariae shedding were observed for 42 days.

RESULTS

Experiment 1: The infection rates and the cercariae production in two isolates of *S. japonicum* are shown in Table 1. Two isolates of *S. japonicum* from Zhejiang and Anhui Province were used to infect *O. hupensis*. After 62-83 days prepatent period, the infection rates were 22.2% and 30.9%,

respectively. Female snails were more susceptible than males. The observation of 34 weeks' time indicated that the isolate from Anhui produced more cercariae than the isolate from Zhejiang. The averages of cercariae production per positive snail per shedding were 13.4 for the Zhejiang isolate and 26.1 for the Anhui isolate. The cercariae production of the two isolates of *S. japonicum* were significantly different ($p < 0.05$). The cercariae production decreased progressively from week to week: higher production in the first three or four weeks, and a reduction in cercariae output every 3-4 weeks. Male positive snails stopped releasing cercariae after 18-19 weeks, but female positive snails continued to shed a few cercariae for 32-33 weeks until they died. The longest life of positive snail was 34 weeks (Fig 1, Table 2).

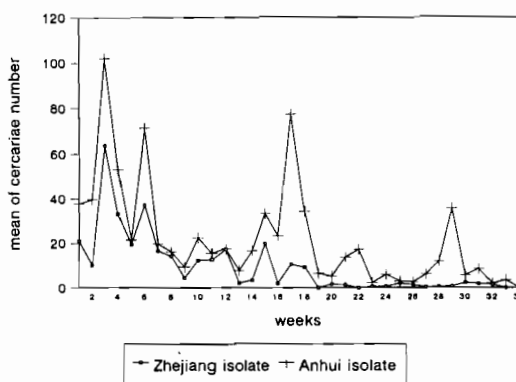


Fig 1—The Dynamics of cercariae shedding for different isolates of *S. japonicum* (Experiment 1).

Table 1

Comparison the infection rates and cercariae production in two isolates of *S. japonicum* (Experiment 1).

Isolate	Infection rate	No. count	Minimum per snail	Maximum per snail	Mean ± SD	Total production
from Zhejiang	Female 28.0% (14/50)	306	0.0	411.0	13.4 ± 41.6	4,098
	Male 16.3% (8/49)	84	0.0	154.0	13.3 ± 27.1	1,115
from Anhui	Female 45.8% (22/48)	545	0.0	467.0	26.6 ± 60.9	14,480
	Male 16.3% (8/49)	106	0.0	287.0	23.6 ± 46.3	2,498

Experiment 2: Initially, all the positive snails in seven groups kept the same level of cercariae production. In the different interval experiments: G1, G2 and G3 released frequently a low number of cercariae with high mortality; G4, G5, G6 and G7 released high numbers of cercariae with low mortality (Table 3, Fig 2). In G1, they shedded

cercariae continually for 20 days. Then there was a reduction in output in every one or two days until the 36th day. Most snails (9/11) stopped shedding cercariae before death. The longest lived in G1 lasted 40 days. Similar results were observed in G2 and G3. In G4, G5, G6 and G7, contrasting results showed higher cercariae production the first three

Table 2

The dynamics of cercariae shedding for different isolates of *S. japonicum* (Experiment 1).

Weeks	Isolates of <i>S. japonicum</i>					
	Zhejiang			Anhui		
	Male	Female	Total	Male	Female	Total
01	4.00	24.00	21.14	25.83	45.67	37.73
02	2.00	11.33	10.00	29.43	43.05	39.64
03	12.25	81.54	63.67	30/75	115.32	102.17
04	17.25	42.14	33.09	44.13	56.37	53.10
05	15.88	21.71	19.59	16.88	23.41	21.67
06	59.80	28.46	37.17	74.14	70.68	71.42
07	27.60	12.46	16.67	13.00	21.74	19.81
08	4.20	18.15	14.28	6.00	18.81	16.30
09	2.20	5.15	4.39	15.00	7.71	9.41
10	2.20	16.31	12.38	14.60	24.52	22.62
11	24.8	8.00	12.67	15.80	15.54	15.67
12	9.25	19.85	17.35	13.25	18.67	17.68
13	0.33	5.33	4.20	10.50	7.11	7.73
14	3.00	3.67	3.53	15.00	17.11	16.81
15	25.67	19.09	20.00	74.50	28.89	33.45
16	0.33	8.73	2.07	8.50	25.16	23.50
17	3.50	12.00	10.58	144.50	69.94	77.35
18	29.00	5.30	9.25	2.00	38.00	34.25
19	0.00	0.00	0.00	0.50	6.94	6.45
20	0.00	2.00	1.64	0.00	5.72	5.20
21	0.00	1.44	1.30	0.00	15.47	13.84
22	0.00	0.00	0.00	0.00	19.24	17.32
23	0.00	0.63	0.55	0.00	2.50	2.35
24	0.00	0.57	0.50	0.00	6.25	5.88
25	0.00	2.29	2.00	0.00	3.06	2.94
26	0.00	1.57	1.25	0.00	2.43	2.53
27	0.00	0.43	0.38	1.00	6.62	6.14
28	0.00	0.67	0.57	0.00	12.92	12.07
29		0.66	0.66	0.00	39.30	35.72
30		2.33	2.33		5.64	5.64
31		1.80	1.80		8.50	8.50
32		1.50	1.50		2.00	2.00
33		0.00	0.00		3.25	3.25
34					0.00	0.00

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times (or weeks). After that, a tendency to decrease occurred from generation to generation. The result was similar to Experiment 1. After 42 days observation, all positive snails in G1 and G2 were dead (70% in G3, but only 50% - 58% positive snails in G4, G5, G6 and G7). Comparing the total cercariae production per snail over 42 days, the production in G1 was the highest and double those in the other experimental groups (Table 4).

temperature below 15°C, but temperature variation of water between 15 - 35°C did not influence the release of cercariae from both *O. quadrasi* and *O. hupensis* (Baumam *et al*, 1948). Light played an important role in *S. japonicum* cercariae release from Chinese snail hosts (Mao *et al*, 1949). Water with pH values ranging from 6.6-7.8 seemed equally suitable for cercariae shedding from *O. hupensis*

Table 3
Comparison of the total cercariae production per and per snail in seven group (Experiment 2).

Group	No. positive snails	Minimum per group	Maximum per group	Mean per snail
Group 1	11	71	1,912	912.7 ± 195.0
Group 2	12	82	796	459.8 ± 71.4
Group 3	10	12	679	465.0 ± 77.0
Group 4	12	148	918	356.2 ± 60.2
Group 5	12	292	897	556.4 ± 51.6
Group 6	12	315	916	587.3 ± 46.5
Group 7	12	209	905	549.2 ± 70.0

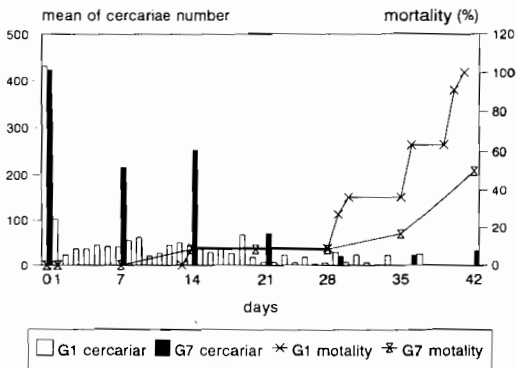


Fig 2-Comparison of the cercariae production and mortality in G 1 and G 7 (Experiment 2).

DISCUSSION

In the early stage studies, the light, temperature and pH value of water were considered to be critical factors in cercariae emergence and production of *S. japonicum*. No shedding was observed at a

and *O. nosophora*. With previous results as background, we designed experiments under identical laboratory conditions: temperature 25°C, a strong light for 3 hours and tap water with pH = 7.0.

Two patterns of the dynamics of cercariae shedding were indicated in the life span of positive snails. The first was a long-time interval and progressive decline pattern. Higher cercariae production occurred in the first three or four weeks, then there was a reduction from week to week, the level of cercariae production alternated between periods of higher and lower 3-4 week productivity. The positive snails shedded cercariae for more than half year. The longest lived lasted 34 weeks. The second was a short-time interval and continuous release pattern. In the every day shedding group, cercariae were released continually for 20 days at the lower production level. However the mortality was higher and all positive snails died in 40 days. In experiment 2, the total cercariae production in per snail in G1 was highest and double that in other groups. It is suggested that shedding cercariae can stimulate cercariae development, higher mortality was attributed to exhaustion of nutrients.

Table 4

The average of cercariae production per snail per shedding for seven group (Experiment 2).

No. day	G1 (11)	G2 (12)	G3 (10)	G4 (12)	G5 (12)	G6 (12)	G7 (12)
0	431.2	468.4	422.9	373.3	372.3	415.8	423.2
1	102.2						
2	21.8	100.2					
3	35.4		116.9				
4	35.5	39.5		115.8			
5	43.7				223.8		
6	40.6	42.7	41.0			208.1	
7	39.7						215.7
8	53.9	48.5		72.9			
9	60.7		109.9				
10	19.4	32.2			151.6		
11	25.5						
12	43.4	18.2	60.2	23.3		128.3	
13	49.0						
14	41.9	59.0					253.1
15	38.3		35.6		88.9		
16	27.6	19.8		43.5			
17	34.6						
18	25.6	8.0	21.3				
19	67.1					177.3	
20	16.7	61.8		23.5	45.7		
21	5.9		11.4				69.8
22	5.7	4.5					
23	21.5						
24	5.3	9.1	35.7	19.8		37.7	
25	17.3				21.4		
26	3.0	4.1					
27	5.0		16.9				
28	28.4	5.9		17.3			18.8
29	6.6						
30	22.1	9.3	18.9		22.4	25.7	
31	5.7						
32	0.9	9.2		41.3			
33	21.1		10.3				
34	0.3	4.3					
35	0.0				10.1		21.8
36	24.0	0.5	1.8	12.0		17.0	
37	0.5						
38	0.5	0.5					
39	0.0		4.6				
40	0.0	0.0		48.8	12.2		
41							
42		0.0	1.0			34.2	30.7
mortality	100%	100%	70%	58%	50%	58%	58%

Based on a series of experiments with *S. haematobium*, *S. intercalatum*, *S. bovis*, *S. mansoni*, and several species of possible intermediate hosts, the total cercariae production and degree of susceptibility were estimated by total cercariae production (TCP)/100 exposed snails (Frandsen, 1979). Because of the amphibious habitat of *Oncomelania* snails, when they are in a dry environment, the snails stop shedding cercariae. Different intervals cause production and mortality variability.

Several factors influenced cercariae shedding and production: different populations of snails, different isolates of parasite, infection rates, snail sex, etc. Significant differences in cercariae production have been reported in different schistosoma species (Theron, 1988, 1989) and in different geographical strains of *S. japonicum* (Theron, 1986). In experiment 1, two isolates of *S. japonicum* from Zhejiang and Anhui Provinces tested were both able to infect *O. hupensis* from Zhejiang Province. However the infection rates were different (22.2% and 30.9%, respectively) and cercariae production gave a significant difference (total production 5,213 and 16,978, respectively). The isolate from Zhejiang had been kept under laboratory conditions for more than 10 years (15 generations). Long time in breeding and laboratory conditions caused population degeneration and weakened cercariae development. On the other hand, infection rates of female snails (28.0-45.8) were higher than of males (16.3). Male positive snails stopped shedding after 18 weeks, but females continued shedding for 33 weeks. Although the averages of cercariae production per male and female snails were the same (13.39-13.26) in the isolate from Zhejiang and 26.57-23.57 in the isolate from Anhui, the total cercariae production was quite different (4,098 and 1,115 in the isolate from Zhejiang, 14,480 and 2,498 in the isolate from Anhui). Variable infection rates were found in different populations of snail hosts. Positive male snails showed higher mortality rates than females. Lower infection rate, higher mortality and early cessation stop of shedding in male snails caused lower levels of cercariae production. Male snails generally were smaller than females.

Xie *et al* (1990, 1992) studied negative conversion of positive snails in Yunnan Province, China. The negative conversion rates were 36.1, 50.0, 41.0, 39.8 and 2.6% in the second, third, fourth, fifth and sixth months after the first shedding, respectively. The present study indicated that only 15.4% (8/52) positive snails in experiment 1 stopped

shedding cercariae 4-10 weeks before death. Most snails shedded the cercariae at low level until death. Some of them stopped for several weeks, then recommenced.

In conclusion, two patterns of the dynamics of cercariae shedding were observed in *S. japonicum* infecting *O. hupensis*. The cercariae production was influenced by several different factors, including *Oncomelania* populations, *Schistosoma* isolates, snail sex, experiment conditions. The studies of dynamics of cercariae emergence and production are advantageous to assessment of the epidemiological situations of schistosomiasis.

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