LABORATORY STUDIES ON HOST-PARASITE RELATIONSHIP OF *BITHYNIA* SNAILS AND THE LIVER FLUKE, *OPISTHORCHIS VIVERRINI*

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Abstract. The infection rate of *Bithynia* snails to *Opisthorchis viverrini* eggs was studied in relation to exposure intensity, age and species of host. It was found that 50 miracidial eggs per snail yielded the highest percentage of living surviving positive snails. *Bithynia funiculata* and *Bithynia siamensis siamensis* were highly susceptible to *O. viverrini*, about four to seven times higher than *Bithynia siamensis goniomphalos*. Young snails, 1-3 months old, appeared more susceptible than old snails.

INTRODUCTION

Liver fluke infection caused by *Opisthorchis viverrini* is endemic in the northeast of Thailand where raw fish is a favorite dish of the local people. This fluke utilizes freshwater fishes, especially cyprinoid fish, as the second intermediate host and *Bithynia* snails as the first intermediate host. Three taxa of *Bithynia* have been reported as the sources of infection in different geographical habitats; *Bithynia funiculata* in the north, *B. siamensis goniomphalos* in the northeast and *B. siamensis siamensis* in the central part of Thailand. The natural infection rates of *O. viverrini* in these snails varied from 0.083 to 1.6% (Wykoff *et al,* 1965; Vajrasthira and Harinasuta, 1966; Upatham and Sukhapanth, 1980; Brockelman *et al,* 1986).

In this study, the infection rate of *Bithynia* snails to *O. viverrini* eggs was determined in relation to exposure intensity, age and species of the host. Life tables of snails will be calculated and survival rates of exposed and unexposed snails will be compared.

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MATERIALS AND METHODS

Snails: Laboratory bred and uninfected field-collected *B. funiculata* (Bf), *B. s. goniomphalos* (Bsg) and *B. s. siamensis* (Bss) were used in this study. Laboratory bred snails (immature) were 1-3 months old, measuring 2-4 mm long and 1.5-2.5 mm wide, while the field-collected snails (mature) measured 6-10 mm long and 4-7 mm wide. The field snails were determined to be free of cercariae through weekly observation for at least three months.

Preparation of *Opisthorchis* eggs: Feces of opisthorchiasis patients were collected, washed several times in tap water by sedimentation, and strained through a 45 μm pore sieve. Eggs in fine fecal materials were left in petri dishes at room temperature (25-29°C) with a change of water daily. Fully developed miracidial eggs were harvested during 1-2 months of cultivation.

Experiments: Infection of snails was carried out individually; the snails were placed singly in tissue culture wells with miracidial eggs. They were left to feed on the eggs for 2 days before transferring to an aquarium. Experimental snails were examined weekly for the presence of opisthorchid cercariae. The numbers of snails shedding cercariae and

dead snails in each group were recorded at weekly intervals for 24 weeks. Dead snails and negative snails after a period of 24 weeks were crushed and examined for rediae and immature cercariae. The snails with those stages were recorded as positive.

Host-parasite relationship of *Bithynia* snails and *O. viverrini* were studied as follows. (1) infection rate and exposure intensity: 30, 50, 90 miracidial eggs were given to each of 70, 24, 60 laboratory-bred Bss snails repectively; (2) infection rate, age and species of host: 115 Bf, 167 Bsg and 156 Bss of lab snails and 40 Bf, 40 Bsg and 47 Bss of field snails were exposed to 90 miracidial eggs per snail (eps); (3) survival of infected snails: life tables of 59 infected (90 eps) and 51 non-infected laboratory snails and 47 infected (90 eps) and 77 non infected field-collected snails were calculated as described by Armitage and Berry (1987).

RESULTS

Exposure intensity and infection rate

The infection rates of laboratory Bss exposed to 30, 50 and 90 eps were 31.4%, 62.5% and 78.3% respectively (Table 1). The number of positive snails increased with the degree of exposure. The infection rate was significantly less in Bss exposed to 30 eps than in those exposed to 50 and 90 eps (X_2 test, $p < 0.025$ and $p < 0.005$, respectively), but there was no significant difference between the infection rates of Bss exposed to 50 and 90 eps.

Age, species of host and infection rate

The infection rates of three taxa of immature laboratory and mature field *Bithynia* snails exposed to 90 eps are shown in Table 2. Laboratory snails

Table 1

Infection rates of laboratory *B.s. siamensis* exposed individually to different numbers of *O. viverrini* eggs.

No. of *O. viverrini* eggs per snail	No. of exposed snails	No. positive snails (%)			X^2 test
		Survived	Dead	Total	
30	70	15 (21.4)	7 (10.0)	22 (31.4)	$p < 0.025$
50	24	11 (45.8)	4 (16.7)	15 (62.5)	
90	60	29 (48.3)	18 (30.0)	47 (78.3)	$p < 0.005$

Table 2

Infection rates of three taxa of laboratory and field *Bithynia* snails exposed to 90 *O. viverrini* eggs per snail.

Snail	No. of exposed snails	No. positive snails (%)		
		Survived	Dead	Total
Laboratory snails				
B. funiculata	115	51 (44.3)	32 (27.8)	83 (72.2)
B.s. goniomphalos	167	8 (4.8)	8 (4.8)	16 (9.6)
B.s. siamensis	156	65 (41.7)	44(26.3)	109 (69.9)
Field snails				
B. funiculata	40	2 (5.0)	17 (42.5)	19 (51.4)
B.s. goniomphalos	40	0	4 (10.0)	4 (10.0)
B.s. siamensis	47	2 (4.2)	17 (36.2)	19 (40.4)

of Bf and Bss were highly susceptible to *O. viverrini* with infection rates of 72.2% and 69.9%, respectively whereas 9.6% of Bsg became infected. The results of field snails showed a similar pattern. High infection rates were found in Bf and Bss (51.4% and 40.4% repectively) whereas that of Bsg was 10%.

Survival rate of infected snails

Life tables of exposed and unexposed laboratory Bss and exposed and unexposed field Bss are shown in Tables 3 and 4, while the percentages of survivors plotted against the duration of exposure are illustrated in Fig 1. Within the first 9 weeks, the percent survival of the exposed laboratory snails was similar to that of unexposed snails. From weeks 9 to 24 the number of living snails in the exposed group decreased with time whereas the number of living non-exposed snails was relatively constant: At the end of week 24 only 24.2% of the snails in the exposed group survived.

The mortality rates of both the exposed and non-exposed field Bss were low in the first eight weeks of the experiment, but increased thereafter. In the beginning of the declining period, the survival rate was similar in both groups of snails, but the percentage of living snails in the exposed group was significantly less than the unexposed control group at the end of the experiment (logrank test, $p < 0.05$).

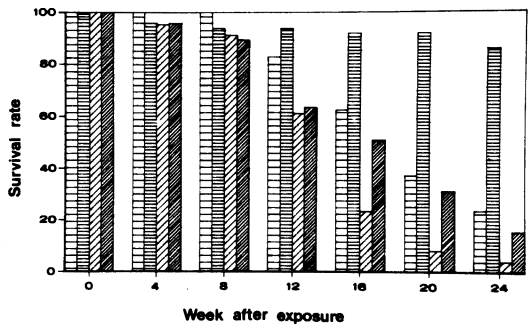

Fig 1—Survival rates of exposed ▤ and unexposed ▩ laboratory *B.s. siamensis* and exposed ▨ and unexposed ▩ field *B.s. siamensis* during 24 weeks of observation. Each snail was exposed to 90 miracidial eggs.

DISCUSSION

The experimental exposure of Bss snails to different number of *O. viverrini* eggs revealed that a fairly high infection rate could be obtained when snails were exposed to 50 eps or more; 30 eps produced only half the number of positive snails

Table 3

Life table of laboratory *B.s. siamensis* exposed and unexposed to *O. viverrini* eggs.

Week after exposure	Unexposed snails					Exposed snails (90 eps)				
	nx	dx	qx	px	lx	nx	dx	qx	px	lx
0 - 4	51	2	0.04	0.96	100	59	0	0	1.00	100
5 - 8	49	1	0.02	0.98	96	59	0	0	1.00	100
9 - 12	48	1	0.02	0.98	94.1	59	10	0.17	0.83	100
13 - 16	47	1	0.02	0.98	92.2	49	12	0.24	0.76	83.0
17 - 20	46	1	0.02	0.98	90.3	37	15	0.40	0.60	62.7
21 - 24	45	2	0.04	0.96	88.5	22	8	0.36	0.64	37.8
25 -					85.0					24.2

nx = number of living snails at the beginning of the experiment.
dx = number of dead snails during this interval.
qx = dx/nx = estimated probability of death.
px = 1 - qx = estimated probability of survival.
lx = 100 p0p1.....px-1 = percentage of survivors after × weeks.

Table 4

Life table of field-collected *B.s. siamensis* exposed and unexposed to *O. viverrini* eggs.

Week after exposure	Unexposed snails					Exposed snails (90 eps)				
	nx	dx	qx	px	lx	nx	dx	qx	px	lx
0 - 4	77	3	0.04	0.96	100	47	2	0.04	0.96	100
5 - 8	74	5	0.07	0.93	96.1	45	2	0.04	0.96	95.7
9 - 12	69	20	0.29	0.71	89.6	43	14	0.33	0.67	91.5
13 - 16	49	10	0.20	0.80	63.6	29	18	0.62	0.38	61.7
17 - 20	39	15	0.38	0.62	50.6	11	7	0.64	0.36	23.4
21 - 24	24	12	0.50	0.50	31.2	4	2	0.50	0.50	8.5
25 -					15.5					4.3

nx = number of living snails at the beginning of the experiment.
dx = number of dead snails during this interval.
qx = dx/nx = estimated probability of death.
px = 1 - qx = estimated probability of survival.
lx = 100 p0p1.....px-1 = percentage of survivors after × weeks.

compared to 50 eps while 90 eps gave a slightly higher infection rate but not significantly different to 50 eps (Table 1). This concurs with Anderson's (1978) explanation that the rate of infection was directly proportional to the density of the infective stages. However, at a certain number of parasites exposed, the infection rate reached its highest since a host of given size could only habor a finite number of invading parasites, whatever the exposure density.

The mortality rate of positive snails was also proportional to the number of miracidial eggs present. This result agreed with Massoud (1974) who reported that the survival rate of snails exposed to a larger number of miracidia was considerably lower than snails exposed to smaller numbers of miracidia. However, Loker (1978) found that the mortality rates of *Lymnaea catascopium* exposed to 3 and 10 miracidia of *Schistosoma douthitti* were not significantly different. Chu *et al* (1966) also reported that the survival rate of *Bulinus truncatus* exposed to small numbers of *S. haematobium* miracidia did not differ significantly from that of snails exposed to larger number of miracidia.

From the results of infection rate and mortality rate of this study, the optimal number of *O. viverrini* eggs for obtaining a high rate of infection and a low mortality rate of infected *Bithynia* snails would be around 50 eps. The percentage of infected living snails obtained from the 50 eps group was as high as that obtained from the 90 eps group, but the mortality rate in infected snails was about half that of the 90 eps groups.

Among the three taxa of *Bithynia* snails, Bsg, which is widely distributed in the liver fluke endemic area, gave the lowest infection rate while Bf and Bss which inhabit non-endemic areas were highly infected with *Opisthorchis* under experimental conditions (Table 2). It seems that Bf and Bss are 4-7 times more susceptible to *O. viverrini* than Bsg. Precautions should therefore be taken in non endemic areas where northeastern people are new settlers.

It is evident that immature laboratory snails were more susceptible to *O. viverrini* infection than mature field snails of the same taxon. This may be accounted for by the difference in age; laboratory snails were, young immature, one to three months old, whereas field snails were mature, and old. *Bithynia* snails become mature and produce offspring at 6 months old and the life span is 2 years (Kruatachue *et al*, 1982). The experimental field-snails were at least 15 months old at the end of the experiments and mortality rates of field snails were expected to be higher than laboratory snails. As it is the estimated probability of survival of laboratory snails were higher than the field snails (Tables 3 and 4).

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REFERENCES

Anderson RM. Population dynamics of snail infection by miracidia. *Parasitol* 1978; 77 : 201-24.

Armitage P, Berry C. Statistical Methods in Medical Research, 2nd ed. London: Blackwell Scientific Publications 1987; pp. 504.

Brockelman WY, Upatham ES, Viyanant V, Ardsungnoen S, Chantanawat R. Field studies on the transmission of the human liver fluke, *Opisthorchis viverrini,* in northeast Thailand. *Int J Parasitol* 1986; 16 : 545-52.

Chu KY, Sabbaghian H, Massoud J. Host-parasite relationship of *Bulinus truncatus* and *Schistosoma haematobium* in Iran. 2. Effect of exposure dosage of miracidia on the biology of the snail host and the development of the parasites. *Bull WHO* 1966; 34 : 121-30.

Kruatachue M, Jantataema S, Ratanatham S, Vichasri S, Upatham ES. A culture method for *Bithynia* (Prosobranchia: Bithyniidae), snail hosts for the trematode *Opisthorchis viverrini. Malacol Rev* 1982; 15 : 63-7.

Loker ES. *Schistosomatium douthitti:* Effect of *Lymnaea catascopium* age on susceptibility to infection. *Exp Parasitol* 1978; 45 : 65-73.

Massond J. The effect of variation in miracidial exposure dose on laboratory infections of *Ornithobilharzia turkestanicum* in *Lymnaea gedrosiana. J Helminthol* 1974; 48 : 139-44.

Upatham ES, Sukhapanth N. Field studies on the bionomics of *Bithynia siamensis siamensis* and the transmission of *Opisthorchis viverrini* in Bangna, Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 1980; 11 : 355-8.

Vajrasthira S, Harinasuta C. Studies on the life cycle, pathology and clinical aspects of the hepatic trematode *Opisthorchis viverrini.* Final Technical Report to US Army Medical Research and Development Command 1966.

Wykoff DE, Harinasuta C, Juttijudata P, Winn MM. *Opisthorchis viverrini* in Thailand - The life cycle and comparison with *O. felineus. J Parasit* 1965; 51 : 207-14.