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Outdoor Observations on Cercarial Formation and Emergence of *Paragonimus ohirai* in Experimentally Infected Snail Hosts

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Abstract

From 1985 to 1987, we carried out experiments on the lung fluke, *Paragonimus ohirai,* to investigate cercarial formation in and emergence from the experimentally infected snail host, *Angustassiminea parasitologica,* kept under outdoor temperatures. The *P. ohirai* miracidia used were derived from eggs expelled by albino rats experimentally infected with the Kiso River (central Japan) strain. It took 40-72 days for miracidia to develop into cercariae in snails infected between April 15 and August 21, but more than 232 days, including a wintering period, in snails infected between August 28 and November 12. In an emergence experiment with daily immersion of snails for 10 days beginning on July 21, cercariae emerged 7 to 10 times from 92.5% of the snails. In an experiment with 9 weekly immersions beginning on August 5, ercariae emerged 6 to 9 times from 55.6% of the snails. In an experiment with 2-weekly immersion for 10 weeks beginning on October 13, the number of emerging cercariae decreased with decline in outdoor temperature, and no cercariae emerged after November 24. Our present experiments under outdoor temperatures suggest that, in the estuary of the Kiso River which is inhabited by *P. ohirai,* the miracidia infecting the snail, *A. parasitologica,* between April and August grow to cercariae within the year, but miracidia infecting after September, and some in late August, grow to cercariae after wintering. It also seems likely that, in the estuary, cercariae emerge frequently whenever the infected snail hosts are immersed in water, but that their emergence from snails ceases after November.

Key words: lung fluke, *Paragonimus ohirai,* cercarial formation, cercarial emergence, 1st intermediate host snail

Introduction

Our previous field studies have reported that *Paragonimus ohirai* is widely distributed in the estuary areas of the Ibi, Nagara and Kiso Rivers, which flow into Ise Bay in central Japan (Matsuo & Makiya 1985, 1987). In these studies, we elucidated that 1) brackish water crabs, especially *Sesarma dehaani,* were highly infected with *P. ohirai* metacercariae at sites 4—13 km away from the estuaries of the 3 rivers, 2) half of the captured Norway rats, *Rattus norvegicus,* were infected with adult worms of *P. ohirai* and 3) snail hosts, *Angustassiminea parasitologica,* were infected with cercariae of the lung fluke. Here we report the results of outdoor observations on cercarial formation in and emergence from the snail, *A. parasitologica,* experimentally infected with miracidia of the Kiso River strain.

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Materials and Methods

The parasite eggs were derived from worm cysts in the lungs of Wistar strain albino rats orally given metacercariae removed from crab hosts, *S. dehaani* from the Kiso River. For miracidia to hatch, the eggs cultivated under outdoor temperatures were put in the refrigerator

(5°C) for 20—30 min and then returned outdoors. Fifty snails, *A. parasitologica,* were exposed *en masse* to 150 miracidia in a petri dish (8.5 cm diameter) with a small amount of water. After 2—3 hour exposure, the 200—300 snails were kept in plastic rearing boxes (25 × 18 cm, and 9 cm depth) half-filled with wet mud from their habitat, and occasionally given small amounts of laver as food on the mud surface and a small amount of water on the bottom of the rearing box via a pipette. The infection of miracidia and cultivation of infected snails were conducted outdoors. The snail hosts used were collected from a site in the Kiso River where the infection rate was very low (0.001%). The collected snails (shell length 3—4 mm) were kept separately in water for about 2 hr prior to infection with miracidia, and only those with no observed cercarial emergence were used for the experiment. Infected snails were sampled at random periodically, and dissected to examine the cercarial formation. Following the detection of redia in snail groups, about 50g of wet mud from each rearing box was examined for cercaria every 10—20 days, but no cercariae were detected in a total of approximately 3 kg. of mud.

Some other snails infected with miracidia in April and kept outdoors were used for cercaria-emergence experiments from August to December 1987. The snails were put individually in a petri dish with a small amount of water at 9 a.m. and the emerging cercariae were counted after 2 hr. The snails were then returned individually to small rearing boxes and repeatedly immersed by the same method in later trials. All the experiments were conducted outdoors, except for the 25°C group. Occasionally, wet mud from each rearing box was examined, but no cercariae were detected.

Results and Discussion

Cercarial formation: Snails from each infected group were dissected to detect sporocysts, rediae and cercariae every 4—20 days. Table 1 summarizes the results before and after cercarial detection in 14 groups of experimentally infected snail hosts from July 7, 1986 to August 21, 1987.

In 9 snail groups (F-N) infected between April 15 and August 21, *P. ohirai* miracidia were observed to grow to cercariae within the year. In group F infected on April 15, rediae but no cercariae were detected on day 65 after miracidial infection (June 19), while cercariae were detected on day 72 (June 26). In groups G, H and I infected between April 28 and June 11, cercariae were detected on day 62—72. In groups J, K and L infected between July 3 and August 7, larval growth accelerated, cercariae being observed on day 40—43. In group N infected on August 21, cercariae detected on day 54 (October 14).

In the other groups A-E infected between August 28 and November 12, larval development was prolonged; the cercarial stage was observed only after wintering. In group A infected on August 28, rediae were detected on day 60 (October 27) and day 258 (May 13), and cercariae were detected on day 272 (May 27). In groups B and C infected in September, rediae were detected within the year; on day 56 (October 31) and day 56 (November 21), respectively. In the following year, rediae were detected on day 262 (May 27) and day 249 (June 2), respectively; cercariae on day 270 (June 2) and day 257 (June 10), respectively. In groups D and E infected in October and November, respectively, sporocysts were detected within the year; on day 67 (December 20) and day 38 (December 20), respectively. In the following year, cercariae were detected on day 258 (June 29) and day 232 (July 2) respectively. Outdoor temperatures under which the 14 snail groups shown in Table 1 were kept as follows: 19.5—35.2°C in July and August 1986, 11.0—30.0°C in September and October, 3.0—21.5°C in November and December, -1.0—16.0°C in January and February 1987, 0.4—25.0°C in March and April, 15.0—32.8°C in May and June, 22.5—34.0°C in July and August, 15.0—33.0°C in September and October.

In similar experiments of 1985—1986 in which 50 snails were exposed *en masse* to 500 miracidia (no data given), in snail groups infected on September 20 and October 1, 1985, cercariae were

Table 1. Summary of cercarial formation of *Paragonimus ohirai* in the experimentally infected snail host, *Angustassiminea parasitologica*, under outdoor temperatures (Results before and after cercarial detection)

Snail group	Date of miracidial infection	Date of snail dissection	Days after infection	No. snails dissected	No. snails positive for rediae	No. snails positive for rediae and cercariae
A	Aug. 28, 1986	May 13, 1987	258	13	11	0
		May 27, 1987	272	3	3	1
		June 2, 1987	278	5	4	2
B	Sep. 5, 1986	May 27, 1987	264	12	10	0
		June 2, 1987	270	4	3	1
		June 10, 1987	278	5	5	2
C	Sep. 26, 1986	June 2, 1987	249	15	12	0
		June 10, 1987	257	5	5	2
		June 18, 1987	265	5	5	1
D	Oct. 14, 1986	June 18, 1987	247	10	8	0
		June 29, 1987	258	2	2	1
		July 14, 1987	273	4	3	1
E	Nov. 12, 1986	June 26, 1987	226	9	6	0
		July 2, 1987	232	5	4	1
		July 23, 1987	253	2	2	1
F	Apr. 15, 1987	June 19, 1987	65	19	15	0
		June 26, 1987	72	10	8	3
		July 1, 1987	77	3	2	2
G	Apr. 28, 1987	June 26, 1987	59	10	9	0
		July 1, 1987	64	5	5	2
		July 14, 1987	77	5	5	2
H	May 12, 1987	July 9, 1987	58	10	10	0
		July 23, 1987	72	5	5	3
I	June 11, 1987	July 23, 1987	42	15	14	0
		Aug. 12, 1987	62	5	5	4
J	July 3, 1987	Aug. 7, 1987	35	15	11	0
		Aug. 12, 1987	40	5	5	4
K	July 7, 1986	Aug. 11, 1986	35	15	13	0
		Aug. 19, 1986	43	3	3	3
L	Aug. 7, 1986	Sep. 10, 1986	34	15	15	0
		Sep. 18, 1986	42	5	5	5
M	Aug. 14, 1987	Sep. 25, 1987	42	15	11	0
		Oct. 7, 1987	54	10	10	5
N	Aug. 21, 1987	Oct. 2, 1987	42	15	10	0
		Oct. 14, 1987	54	24	15	2

detected on day 299 (July 16, 1986) and day 295 (July 23), respectively. In a snail group infected on April 25, 1986, cercariae were detected on day 74 (July 8 of the same year).

Concerning the results for the snail groups A and N infected in late August, the miracidia infecting snails on August 21, 1987 (group N) grew to cercariae within the year. The miracidia infecting snails on August 28, 1986 (group A) grew to rediae within the year, and to cercariae the following May after wintering. It is suggested that outdoor temperatures from late August critically

influence cercarial formation, although present results are insufficient for discussion of the relationship between rearing temperature and cercarial formation.

The present experiments were carried out on the university campus located about 30 km east of the Kiso River estuary inhabited by *P. ohirai*. Since the same climatic conditions prevail in both areas, it is suggested that in the Kiso River area, *P. ohirai* miracidia infecting the snail host after September, and some in late August, grow to sporocysts or rediae within the year, and to cercariae after wintering.

Cercarial emergence: The experiments on cercarial emergence were conducted under outdoor temperatures, except for the 25°C snail group. Table 2 summarizes the results of the outdoor experiment with daily immersion for 10 days from July 21 to July 30. At the 1st and final immersions, cercariae emerged from 40 and 31 out of 40 snails, respectively, and the mean number of cercariae emerging per snail was 100.9 and 26.3, respectively. The frequency of cercarial emergence from individual snails was as follows (no data given): emergence was observed 10 times in 10 snails (25.0%), 7—9 times in 27 snails (67.5%) and 5—6 times in 3 snails (7.5%). Immediately after the experiment, all snails were dissected and shown to harbor *P. ohirai* rediae and cercariae. The outdoor temperature during this experiment was 26.0—35.0°C.

Table 3 summarizes the results of the outdoor

Table 2. Summary of emergence experiments of *Paragonimus ohirai* cercariae from experimentally infected snails, *Angustassiminea parasitologica*, under outdoor temperatures (10 daily observations from July 21 to 30, 1987 using 40 snails infected with miracidia in April, 1987)

Observation no.	1	2	3	4	5	6	7	8	9	10
Date of observation	July 21	July 22	July 23	July 24	July 25	July 26	July 27	July 28	July 29	July 30
No. snails releasing cercariae	40	18	26	39	31	37	37	39	39	31
No. cercariae emerging from each snail	9-406	1-203	1-264	1-263	1-135	3-304	2-271	1-248	2-102	1-140
Mean no. cercariae emerging per snail	100.9	20.7	45.7	64.6	36.6	112.9	54.1	87.7	38.6	26.3

Table 3. Summary of emergence experiments of *Paragonimus ohirai* cercariae from experimentally infected snails, *Angustassiminea parasitologica*, under outdoor temperatures (9 weekly observations from August 5 to September 30, 1987 using 34 snails infected with miracidia in April, 1987)

Observation no.	1	2	3	4	5	6	7	8	9
Date of observation	Aug. 5	Aug. 12	Aug. 19	Aug. 26	Sept. 2	Sept. 9	Sept. 16	Sept. 23	Sept. 30
No. snails releasing cercariae	34	30	22	19	29	24	21	10	11
No. cercariae emerging from each snail	156-1025	2-390	1-425	1-232	9-154	1-104	1-51	1-115	3-70
Mean no. cercariae emerging per snail	583.3	147.5	74.7	83.0	54.6	37.5	10.0	20.6	16.0

Table 4. Summary of emergence experiments of *Paragonimus ohirai* cercariae from experimentally infected *Angustassiminea parasitologica* under outdoor temperatures and a constant temperature of 25°C (6 2-weekly observations from October 13 to December 22, 1987 using snails divided into 2 groups after the experiment shown in Table 3)

Observation no.	1		2		3		4		5		6	
Date of observation	Oct. 13		Oct. 27		Nov. 10		Nov. 24		Dec. 8		Dec. 22	
Snail-rearing temperature	outdoor	25°C	outdoor	25°C	outdoor	25°C	outdoor	25°C	outdoor	25°C	outdoor	25°C
No. snails releasing cercariae /No. snails examined	5/17	7/17	11/17	4/17	5/17	6/16	0/17	4/16	0/17	5/14	0/17	5/14
No. cercariae emerging from each snail	3-251	15-367	1-87	8-71	1-13	2-196	—	5-92	—	9-50	—	5-83
Mean no. cercariae emerging from per snail	81.6	85.7	24.8	39.5	6.0	75.2	—	45.3	—	32.6	—	41.6

experiment with 9 weekly immersions from August 5 to September 30. At the 1st and final immersions, cercariae emerged from 34 and 11 out of 34 snails, respectively, and the mean number of cercariae emerging per snail was 583.3 and 16.0, respectively. The frequency of cercarial emergence from individual snails was as follows (no data given): emergence was observed 9 times in 1 snail (2.9%), 5—8 times in 25 snails (73.5%), and 2—4 times in 9 snails (23.5%). The outdoor temperature during this experiment was 25.0—34.0°C during August, but it fell to 20.5—26.0°C on the day of the final immersion (September 30). Then, on September 30, the snails were divided into 2 groups: one was remained outdoors and the other was incubated at a constant temperature of 25°C. The emergence experiment with 6 2-weekly immersions of the 2 groups was conducted outdoors and at 25°C from October 13 to December 22. As summarized in Table 4, cercarial emergence in the outdoor group was observed between the 1st and the 3rd immersions (outdoor temperature: 8.0—28.0°C), but no cercariae were observed to emerge between the 4th and 6th immersions (outdoor temperature: 2.0—17.0°C). On dissection of snails at the end of the experiment, *P. ohirai* rediae were detected in the snails, but no cercariae. In the 25°C group, cercarial emergence was observed in 23.0—41.2% of the snails at each immersion, the mean number of cercariae emerging per snail being 32.6—85.7. On dissection at the end of the experiment, cercariae were detected together with rediae in 5 out of 14 snails, while rediae alone were detected in 5 snails.

Yoshida (1961) reported on cercarial emergence of *P. ohirai* from the snail host, *A. parasitologica,* but observations were limited to the daily immersion of 2 snails for 4 days. Emergence was seen 3 and 4 times in respective snails. In the present study, many snails infected with miracidia were used and cercarial emergence from experimentally infected snails was observed under outdoor temperatures from July to December. We established that many cercariae frequently emerge from the infected snails whenever the snails are immersed in water and that the number of cercaria-releasing snails and the mean number of cercariae emerging per snail decreases steeply after mid-September with the fall in outdoor temperature. The present results suggest that cercarial formation and emergence

ceases under outdoor temperatures after November.

Our present results suggest that, in the estuary of the Kiso River inhabited by *P. ohirai* 1) *P. ohirai* miracidia infecting the snail hosts between April and August grow to cercariae within the year, but miracidia infecting the snails after September, and some in late August, grow to cercariae after wintering; 2) cercariae frequently emerge from infected snails whenever the snails are immersed in water, but cercarial formation ceases around November.

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