นิพนธ์ต้นฉบับ ▪ Original Article

การผันแปรของฤดูกาลต่อความชุกของการติดเชื้อตัวอ่อนระยะเซอร์คาเรียของพยาธิใบไม้ในหอยน้ำจืด *Bithynia siamensis goniomphalos* ในนาปรัง จังหวัดขอนแก่น ประเทศไทย

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Seasonal Fluctuation of Cercarial Trematode Infections in Freshwater Snails of *Bithynia Siamensis Goniomphalos* from In-And Out-Season Rice Paddy Fields, Khon Kaen Province, Thailand

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หลักการและวัตถุประสงค์: การติดเชื้อพยาธิใบไม้เป็นปัญหาที่สำคัญทางระบบสาธารณสุขของประเทศไทย วัตถุประสงค์ของการศึกษานี้เพื่อศึกษาอัตราความชุกของการติดเชื้อตัวอ่อนระยะเซอร์คาเรียของพยาธิใบไม้ในหอย *Bithynia siamensis goniomphalos* และวิเคราะห์ความสัมพันธ์ระหว่างการติดเชื้อและปัจจัยต่าง ๆ

วิธีการศึกษา: การศึกษาความชุกของการติดเชื้อตัวอ่อนระยะเซอร์คาเรียของหอย *B. siamensis goniomphalos* สุ่มเก็บตัวอย่างในนาปรัง 2 แหล่งพื้นที่จังหวัดขอนแก่น (มกราคม 2557-กุมภาพันธ์ 2558)

ผลการศึกษา: พบหอยติดเชื้อพยาธิใบไม้ร้อยละ 2.82 (พบระยะเซอร์คาเรีย 14 กลุ่ม) กลุ่มที่มีความชุกของการติดเชื้อมากที่สุดคือ Xiphidiocercariae ชนิด virgulate 2 ส่วนในนาปรังแหล่งที่ 2 พบหอยที่ติดเชื้อร้อยละ 2.67 (พบระยะเซอร์คาเรีย 11 กลุ่ม) หอยเพศเมียมีความชุกของการติดเชื้อกลุ่ม Ophthalmo xiphidiocercariae และ Pleurolophocercous มากกว่าหอยเพศผู้ หอยขนาดใหญ่มีความชุกของการติดเชื้อ กลุ่ม Xiphidiocercariae (ชนิด virgulate 1 2 และ 3) Ophthalmoxiphidiocercariae และ Pleurolophocercous มากกว่าหอยขนาดกลาง พบความชุกของการติดเชื้อ Xiphidiocercariae ชนิด virgulate 2 Ophthalmoxiphidiocercariae และ Pleurolophocercous มากกว่าหอยที่พบในฤดูร้อน ในฤดูหนาวพบความชุกของการติดเชื้อ Xiphidiocercariae ชนิด virgulate 2 Xiphidiocercariae ชนิด virgulate 3 Ophthal-

Background and Objective: Trematode infections have been considered as important public health in Thailand. This study aimed to investigate the prevalence of trematode infections in *Bithynia siamensis goniomphalos* snails and analyze the association between prevalence of trematode infections with many factors.

Method: Cercarial infections in *B. siamensis goniomphalos* were studied in 2 localities of in- and out-season rice paddy fields in Khon Kaen Province (January 2014-February 2015).

Results: The average prevalence of cercarial infections in snails during the studying period was 2.82% (14 types of cercariae) in locality 1 and 2.67% (11 types of cercariae) in locality 2. The prevalence of cercarial infections with Ophthalmoxiphidiocercariae and Pleurolophocercous in female were higher than male snail. The prevalence of cercarial infections with Xiphidiocercariae (type virgulate 1, 2, and 3), Ophthalmoxiphidiocercariae, and Pleurolophocercous in large-sized snails were higher than medium and small-sized snails. Snails which were found in rainy season were higher prevalence of infection with Xiphidiocercariae type virgulate 2, Ophthalmoxiphi-

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moxiphidiocercariae และ Pleurolophocercous มากกว่าหอยที่พบในฤดูร้อน แต่ปัจจัยของ อุณหภูมิ ปริมาณน้ำฝน และความเค็มต่อความชุกของการติดเชื้อ พบว่า ไม่มีนัยสำคัญทางสถิติ

สรุป: หอย *B. siamensis goniomphalos* สามารถเป็นโฮสต์กลางพยาธิใบไม้ได้หลายชนิด หอยเพศเมีย และหอยขนาดใหญ่มีโอกาสการติดเชื้อพยาธิใบไม้สูงกว่าหอยเพศผู้และหอยขนาดกลาง

คำสำคัญ: ความชุกของการติดเชื้อตัวอ่อนระยะเซอร์คาเรีย, หอย *Bithynia siamensis goniomphalos*, นาปรัง

diocercariae and Pleurolophocercous than hot-dry season. Snails which were found in cool-dry season were higher prevalence of infection with Xiphidiocercariae (type virgulate 2 and 3) Ophthalmoxiphidiocercariae, and Pleurolophocercous than hot-dry season. But the prevalence of cercarial infections in the correlation factors of mean daily air temperature, average monthly rainfall, and salinity were not different significantly.

Conclusions: *Bithynia siamensis goniomphalos* snails served as intermediate host of various trematodes. Female snail was higher prevalence of infection than male snail. Large-sized snails were higher prevalence of infection than were medium-sized snails.

Keywords: cercarial infection, *Bithynia siamensis goniomphalos*, in- and out-season rice paddy field

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Introduction

Bithynia siamensis goniomphalos snails serve as intermediate host of several trematodes, including Amphistome cercariae (Superfamily Paraphistomatoidea), Echinostome cercariae (Superfamily Echinostomatoidea), Furcocercous cercariae (Superfamily Strigeoidea and Superfamily Schistosomatoidea), Pleurolophocercous cercariae (Superfamily Opisthorchiodea), Monostome cercariae (Superfamily Notcotyloidea), Gymnocephalous cercariae (Superfamily Echinostomatoidea), Xiphidiocercariae (Superfamily Plagiorchiodea).[1-6]

Several studies reported that the prevalence of cercarial trematode infections fluctuated depending on season. For instance, the study of seasonal transmission patterns of *Opisthorchis viverrini sensu lato* (s.l.) and virgulate cercaria (Family Lecithodendriidae) in *B. siamensis goniomphalos* snails, the prevalence of both trematodes varied significantly with season which the highest in rainy season followed by cool-dry and hot-dry seasons.[7] Thus, the *B. siamensis goniomphalos* snails might play important role in order to carry life cycles of variety of trematodes and also spread the trematodes to other second intermediate / or other definitive animals and human hosts. The prevalence of cercarial trematode infections in the *B. siamensis goniomphalos* snails might fluctuate relate to many factors. The peak of high prevalence of trematode infections might be baseline data for further studies for more information to control and eliminate this intermediate host and trematodes.

This study tends to find out the factors including gender and size of snails, rainfall, air temperature, water quality and seasons that might reflect to the fluctuation of prevalence of trematode infections in *B. siamensis goniomphalos* snails in in- and out-season rice paddy fields.

Materials and Methods

1. Snail sample collection

Cercarial trematode infections in fresh water snails, *B. siamensis goniomphalos* were studied in 2 localities (locality1 located at 16.457773 ° N latitude and 102.866404° E longitude, locality 2 located at 16.439076° N latitude and 102.941727 ° E longitude) of in- and out-season rice paddy fields in Mueang Khon Kaen District, Khon Kaen Province, northeast Thailand. The snails were collected once a month during January 2014-February 2015. The sampling was done by manual collection or using wire-mesh scoops where aquatic plants were present. Water physicochemical parameters were measured including water temperature (at 25cm depth), dissolved oxygen, conductivity, pH and salinity using a radiometer (90-FL model, TPS PTY. Ltd., Brisbane, Australia). Monthly data of average rainfall and average daily temperatures (°C, average temperature of day time and night time) were obtained from Northeastern Meteorological Center, Mueang Khon Kaen District, Khon Kaen

Province. The collected snails were washed and transported to the laboratory (Department of Parasitology, Khon Kaen University) in porous plastic bags on the day of collection. All snails were identified as *B. siamensis goniomphalos* based on the morphological characters of the shell and operculum following available keys and descriptions.[8-9] Snails were counted and sorted according to shell size (small<5mm, medium 5-8 mm, large>8mm) and gender prior to examination for trematode infections.

2. Examination for trematode infections

Cercarial trematode infection in *B. siamensis goniomphalos* was detected using the cercarial shedding method and observe under dissecting microscope (40X). Individual snails were washed and separated into a 5 ml plastic cup containing 5 ml de-chlorinated tap-water and covered with a porous lid to prevent the snail from escaping from the containers. Cercarial shedding was stimulated by exposure to electric light (daylight) for 3-4 h at room temperature (25 ± 2°C). Cercarial shedding was done three times a month for consecutive two months in the case of any individual from which cercaria had not emerged. Cercarial identification was done in fresh preparations after staining with 0.1% neutral red and observation under a microscope and camera lucida drawing. Cercarial trematode identification was based on morphology including eyespots, body, tail, oral sucker, ventral sucker, stylet, pharynx, penetration gland and excretory bladder following available descriptions.[1, 6, 10-12]

3. Statistical analyses

Statistical analyses were done using SPSS v. 16.0. The multiple logistic regression by adjusted odds ratios (OR) was used to test the prevalence of trematode infections in relation to snail gender and size, and the influent factors of average monthly rainfall, average daily temperature, water temperature and salinity. Any $p < 0.05$ was regarded as significant difference.

Results

1. Prevalence of trematode infections

In locality 1, among the total of 36,214 *B. siamensis goniomphalos* snails, 2.82% (1,020/36,214) were infected with trematode. Fourteen morphologically types of cercariae were found, including Xiphidiocercariae type virgulate 2 (0.92%), Xiphidiocercariae type virgulate 3 (0.73%), Xiphidiocercariae type virgulate 1 (0.52%), Ophthalmoxiphidiocercariae (0.22%), Pleurolophocercous (*O. viverrini*) (0.20%), Parapleurolophocercous (0.06%), Cystophorouscercariae type 2 (0.05%), Brevifurcate-pharyngeate type 1 (0.04%), Amphistomes (0.03%), Monostomes (0.02%), Longifurcate-pharyngeate type 1 (0.02%), Longifurcate-pharyngeate type 2 (0.01%) and Cystophorouscercariae type 1 (0.01%). In locality 2, the overall prevalence of trematode infection in *B. siamensis goniomphalos* was 2.67% (428/16,046). In descending order of prevalences, 11 types of cercariae were detected: Ophthalmoxiphidiocercariae (0.86%), Xiphidiocercariae type virgulate 2 (0.63%), Xiphidiocercariae type virgulate 3 (0.59%), Xiphidiocercariae type virgulate 1 (0.25%), Pleurolophocercous (*O. viverrini*) (0.17%), Cystophorous-cercariae type 2 (0.07%), Parapleurolophocercous (0.03%), Longifurcate-pharyngeate type 2 (0.02%), Brevifurcate-pharyngeate type 2 (0.02%), Cystophorouscercariae type 1 (0.01%), and Amphistomes (0.01%) (Figure 1 and Table 1).

2. The associations between gender of snail hosts and the prevalence of trematode infections in *Bithynia siamensis goniomphalos* snails

In locality 1, female snails were higher prevalence of infections with Ophthalmoxiphidiocercariae and Pleurolophocercous (*O. viverrini*) cercariae than male snails with 0.58 times (95 % CI = 0.37, 0.92, p = 0.02) and 1.64 times (95 % CI = 1.00, 2.62, p = 0.04), respectively (Table 2). Similarly, in locality 2, female snails were higher prevalence of infection with Ophthalmoxiphidiocercariae than male snails, 0.65 times (95 % CI = 0.46, 0.92, p = 0.02). There is no significant difference in another cercarial type (Table 3).

3. The associations between shell size of snail hosts and the prevalence of trematode infections in *Bithynia siamensis goniomphalos* snails

In locality 1, the large size snails were higher prevalence of infections with cercarial types including Xiphidiocercariae type virgulate 2, Xiphidiocercariae type virgulate 3, Xiphidiocercariae type virgulate 1, and Ophthalmoxiphidiocercariae than medium size snails, 2.76, 3.33, 4.83 and 15.8 times, respectively (Table 2).

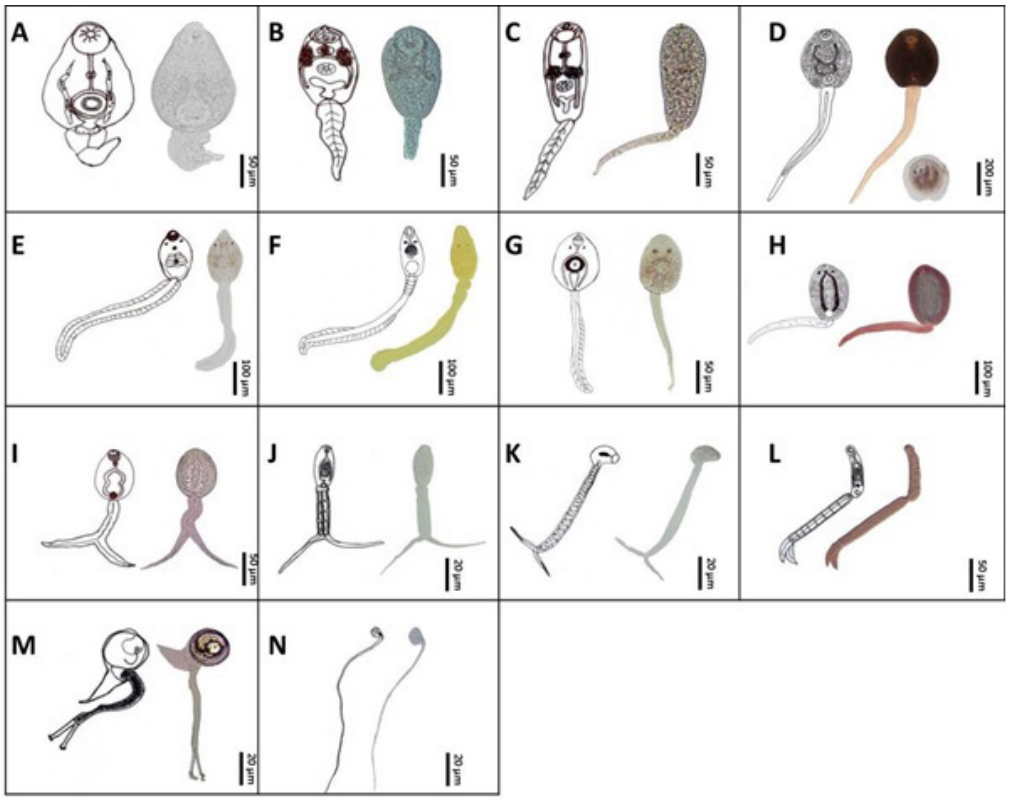

Figure 1 Type of cercariae released from *Bithynia siamensis goniomphalos*;
A. Xiphidiocercariae type virgulate 1 B. Xiphidiocercariae type virgulate 2 C. Xiphidiocercariae type virgulate 3
D. Amphistomes E. Parapleurolophocercous F. Pleurolophocercous (*Opisthorchis viverrini*)
G. Ophthalmoxiphidiocercariae H. Monostomes I. Longifurcate-pharyngeate type 1
J. Longifurcate-pharyngeate type 2 K. Brevifurcate-pharyngeate type 1 L. Brevifurcate-pharyngeate type 2
M. Cystophorouscercariae type 1 N. Cystophorouscercariae type 2

Table 1 Prevalence of cercarial infection in *Bithynia siamensis goniomphalos* snails in locality 1 and 2

Code	Cercarial types	Prevalence of cercarial infection (number of snails)	
		Locality 1	Locality 2
A	Xiphidiocercariae type virgulate 1	0.52 (188)	0.25 (40)
B	Xiphidiocercariae type virgulate 2	0.92 (332)	0.63 (101)
C	Xiphidiocercariae type virgulate 3	0.73 (263)	0.59 (94)
D	Amphistomes	0.03 (10)	0.01 (2)
E	Parapleurolophocercous	0.06 (20)	0.03 (5)
F	Pleurolophocercous (*O. viverrini*)	0.20 (72)	0.17 (28)
G	Ophthalmoxiphidiocercariae	0.22 (79)	0.86 (138)
H	Monostomes	0.02 (6)	0
I	Longifurcate-pharyngeate type 1	0.02 (7)	0
J	Longifurcate-pharyngeate type 2	0.01 (2)	0.02 (3)
K	Brevifurcate-pharyngeate type 1	0.04 (16)	0
L	Brevifurcate-pharyngeate type 2	0.01 (3)	0.02 (4)
M	Cystophorouscercariae type 1	0.01 (3)	0.01 (2)
N	Cystophorouscercariae type 2	0.05 (19)	0.07 (11)
	Total	**2.82 (1,020/36,214)**	**2.67 (428/16,046)**

In locality 2, the large size snails were higher prevalence of infections with cercarial types of Ophthalmoxiphidiocercariae, Xiphidiocercariae type virgulate 2, Xiphidiocercariae type virgulate 3, Xiphidiocercariae type virgulate 1, and Pleurolophocercous (*O. viverrini*) than medium size snails 66.73, 2.32, 2.91, 3.15 and 30.04 times, respectively (Table 3).

4. The associations between seasons and the prevalence of trematode infections in *Bithynia siamensis goniomphalos* snails

In locality 1, the snails which were found in rainy season, were higher prevalence of infections with cercarial types including Xiphidiocercariae type virgulate 2, Ophthalmoxiphidiocercariae and Pleurolophocercous (*O. viverrini*) than hot-dry season for 1.43, 0.19, 1.52 times, respectively. And the snails which found in cool-dry season were higher prevalence of infections with cercarial types of Xiphidiocercariae type virgulate 2, Xiphidiocercariae type virgulate 3, Ophthalmoxiphidiocercariae and Pleurolophocercous (*O. viverrini*) for 2.39, 1.51, 0.26 and 0.28 times, respectively (Table 2).

In locality 2, the snails which were found in rainy season, were higher prevalence of infections with cercarial types of Ophthalmoxiphidiocercariae and Pleurolophocercous (*O. viverrini*) than hot-dry season,

Table 2 Odds ratio of top 5 prevalence of cercarial infection in *Bithynia siamensis goniomphalos* snail and other factors in locality 1

Cercarial types (% infection)	Factors	Locality 1			
		No. infected snails	Total snails	Adjusted OR (95% CI)	P-value
	Genders				
	- Male	142	16,996	Reference	-
	- Female	190	19,218	1.22 (0.98-1.52)	0.07
	Sizes				
Xiphidiocercariae	- Small	0	211	-	-
type virgulate 2	- Medium	84	16,978	Reference	-
(0.92%)	- Large	248	19,025	2.76 (2.15-3.54)	0.00*
	Seasons				
	- Rainy	86	9,835	1.43 (1.06-1.91)	0.02*
	- Cool-dry	148	10,538	2.39 (1.85-3.09)	0.00*
	- Hot-dry	98	15,841	Reference	-
	Genders				
	- Male	118	16,996	Reference	-
	- Female	145	19,218	1.13 (0.88-1.44)	0.33
	Sizes				
Xiphidiocercariae	- Small	0	211	-	-
type virgulate 3	- Medium	57	16,978	Reference	-
(0.73%)	- Large	206	19,025	3.33 (2.48-4.46)	0.00*
	Seasons				
	- Rainy	56	9,835	0.86 (0.62-1.19)	0.35
	- Cool-dry	101	10,538	1.51 (1.15-1.99)	0.00*
	- Hot-dry	106	15,841	Reference	-

*OR odds ratio, CI confidence interval, *Statistically significant correlation ($p<0.05$)

Table 2 Odds ratio of top 5 prevalence of cercarial infection in *Bithynia siamensis goniomphalos* snail and other factors in locality 1 (cont.)

Cercarial types (% infection)	Factors	Locality 1			
		No. infected snails	Total snails	Adjusted OR (95% CI)	P-value
	Genders				
	- Male	101	16,996	Reference	-
	- Female	87	19,218	0.79 (0.59-1.05)	0.11
	Sizes				
Xiphidiocercariae	- Small	1	211	-	-
type virgulate 1	- Medium	29	16,978	Reference	-
(0.52%)	- Large	158	19,025	4.83 (3.25-7.18)	0.00*
	Seasons				
	- Rainy	43	9,835	0.73 (0.51-1.04)	0.09
	- Cool-dry	49	10,538	0.82 (0.58-1.15)	0.26
	- Hot-dry	96	15,841	Reference	-
	Genders				
	- Male	49	16,996	Reference	-
	- Female	30	19,218	0.58 (0.37-0.92)	0.02*
	Sizes				
Ophthalmo-	- Small	0	211	-	-
xiphidiocercariae	- Medium	4	16,978	Reference	-
(0.22%)	- Large	75	19,025	15.8 (5.78-43.24)	0.00*
	Seasons				
	- Rainy	7	9,835	0.19 (0.08-0.40)	0.00*
	- Cool-dry	10	10,538	0.26 (0.14-0.52)	0.00*
	- Hot-dry	62	15,841	Reference	-
	Genders				
	- Male	26	16,996	Reference	-
	- Female	46	19,218	1.64 (1.00-2.62)	0.04*
	Sizes				
Pleurolophocercous	- Small	0	211	-	-
(*O. viverrini*)	- Medium	14	16,978	Reference	-
(0.20%)	- Large	58	19,025	3.63 (2.02-6.50)	0.09
	Seasons				
	- Rainy	32	9,835	1.52 (0.94-2.47)	0.00*
	- Cool-dry	6	10,538	0.28 (0.12-0.66)	0.00*
	- Hot-dry	34	15,841	Reference	-

*OR odds ratio, CI confidence interval, *Statistically significant correlation ($p<0.05$)

Table 3 Odds ratio of top 5 prevalence of cercarial infection in *Bithynia siamensis goniomphalos* snail and other factors in locality 2

Cercarial types (% infection)	Factors	Locality 2			
		No. infected snails	Total snails	Adjusted OR (95% CI)	P-value
Ophthalmo-xiphidiocercariae (0.86%)	**Genders**				
	- Male	88	7,500	Reference	-
	- Female	50	8,546	0.65 (0.46-0.92)	0.02*
	Sizes				
	- Small	0	112	-	-
	- Medium	2	7,742	Reference	-
	- Large	136	8,192	66.73 (16.49-269.96)	0.00*
	Seasons				
	- Rainy	8	4,128	0.10 (0.05-0.21)	0.00*
	- Cool-dry	5	4,540	0.06 (0.02-0.15)	0.00*
	- Hot-dry	125	7,378	Reference	-
Xiphidiocercariae type virgulate 2 (0.63%)	**Genders**				
	- Male	49	7,500	Reference	-
	- Female	52	8,546	0.95 (0.64-1.41)	0.79
	Sizes				
	- Small	0	112	-	-
	- Medium	29	7,742	Reference	-
	- Large	72	8,192	2.32 (1.50-3.57)	0.00*
	Seasons				
	- Rainy	17	4,128	0.97 (0.53-1.75)	0.91
	- Cool-dry	54	4,540	2.83 (1.81-4.44)	0.00*
	- Hot-dry	30	7,378	Reference	-
Xiphidiocercariae type virgulate 3 (0.59%)	**Genders**				
	- Male	46	7,500	Reference	-
	- Female	48	8,546	0.96 (0.64-1.44)	0.84
	Sizes				
	- Small	0	112	-	-
	- Medium	23	7,742	Reference	-
	- Large	71	8,192	2.91 (1.81-4.66)	0.00*
	Seasons				
	- Rainy	23	4,128	1.08 (0.64-1.83)	0.77
	- Cool-dry	35	4,540	1.51 (0.95-2.42)	0.08
	- Hot-dry	36	7,378	Reference	-

*OR odds ratio, CI confidence interval, *Statistically significant correlation ($p<0.05$)

Table 3 Odds ratio of top 5 prevalence of cercarial infection in *Bithynia siamensis goniomphalos* snail and other factors in locality 2 (cont.)

Cercarial types (% infection)	Factors	Locality 2			
		No. infected snails	Total snails	Adjusted OR (95% CI)	P-value
	Genders				
	- Male	17	7,500	Reference	-
	- Female	23	8,546	1.23 (0.65-2.31)	0.52
	Sizes				
Xiphidiocercariae	- Small	1	112	-	-
type virgulate 1	- Medium	9	7,742	Reference	-
(0.25%)	- Large	30	8,192	3.15 (1.45-6.65)	0.00*
	Seasons				
	- Rainy	18	4,128	2.01 (1.01-4.00)	0.45
	- Cool-dry	6	4,540	0.61 (0.24-1.57)	0.30
	- Hot-dry	16	7,378	Reference	-
	Genders				
	- Male	11	7,500	Reference	-
	- Female	17	8,546	1.79 (0.83-3.83)	0.13
	Sizes				
Pleurolophocercous	- Small	0	112	-	-
(*O. viverrini*)	- Medium	1	7,742	Reference	-
(0.17%)	- Large	27	8,192	30.04 (4.07-221.80)	0.00*
	Seasons				
	- Rainy	1	4,128	0.06 (0.00-0.44)	0.00*
	- Cool-dry	1	4,540	0.05 (0.00-0.4)	0.00*
	- Hot-dry	26	7,378	Reference	-

*OR odds ratio, CI confidence interval, *Statistically significant correlation (p<0.05)

0.10 and 0.06 times, respectively. And the snails which were found in cool-dry season, were higher prevalence of infections with cercarial types of Ophthalmoxiphidiocercariae, Xiphidiocercariae type virgulate 2 and Pleurolophocercous (*O. viverrini*) than hot-dry season for 0.06, 2.83 and 0.05 times, respectively (Table 3).

5. The associations between physical factors and the prevalence of trematode infections in *Bithynia siamensis goniomphalos* snails

The average monthly rainfall and the average daily air temperature of Mueang Khon Kaen District were 2.66 (0 - 8.60) mm and 27.5 (21.67 - 29.83) °C. The average salinity of locality 1 and 2 were 329 (80 - 504) ppm and 380 (105-534) ppm, respectively. The factors of the average monthly rainfall, the average daily air temperature, and the salinity was not significantly different associated to prevalence of any trematode infection in both 2 localities (p >0.05) (Table 4).

6. Seasonal fluctuation of prevalence of cercarial trematode infections in *Bithynia siamensis goniomphalos* snails

This study revealed that the *B. siamensis goniomphalos* snails carried high prevalence trematodes of infections, of top five cercarial types

Table 4 Odds ratio of prevalence of cercarial infection in *Bithynia siamensis goniomphalos* snail correlated to variety factors in locality 1 and 2

Factors	Locality 1			Locality 2		
	Prevalence of cercarial infection (positive/total)	OR (95% CI)	P-value	Prevalence of cercarial infection (positive/total)	OR (95% CI)	P-value
Genders						
Male (reference)	2.85 (485/16,996)	-	-	3.03 (227/7,500)	-	-
Female	2.78 (535/19,218)	1.01 (0.89-1.15)	0.81	2.35 (201/8,546)	0.87 (0.72-1.06)	0.17
Sizes						
Small (reference)	0.47 (1/211)	-	-	0.89 (1/112)	-	-
Medium	1.23 (209/16,978)	3.17 (0.44-22.8)	0.25	0.86 (67/7,742)	2.1 (0.29-15.34)	0.46
Large	4.26 (810/19,025)	11.42 (1.59-81.93)	0.01*	4.39 (360/8,192)	13.17 (1.82-95.44)	0.01*
Seasons						
Cool-dry (reference)	3.28 (346/10,538)	-	-	2.36 (107/4,540)	-	-
Rainy	2.46 (242/9,835)	2.05 (1.41-2.96)	0.00*	1.69 (70/4,128)	1.48 (0.85-2.59)	0.16
Hot-dry	2.73 (432/15,841)	3.77 (2.32-6.13)	0.00*	3.40 (251/7,378)	1.75 (.85-3.62)	0.13
Total	2.82 (1,020/36,214)			2.67 (428/16,046)		
	Average (range)	**OR (95% CI)**	**P-value**	**Average (range)**	**OR (95% CI)**	**P-value**
1.Salinity (ppm)	329 (80-504)	1.00 (0.99-0.99)	0.00	380 (105-534)	1.00 (0.99-1.00)	0.83
2.Mean daily air temperature (°C)	27.5 (21.67 - 29.83)	1.11 (0.99-1.26)	0.08			
3.Average monthly rainfall (mm)	2.66 (0 - 8.60)	0.74 (0.69-0.79)	0.00			

*OR odds ratio, CI confidence interval, *Statistically significant correlation (P<0.05)

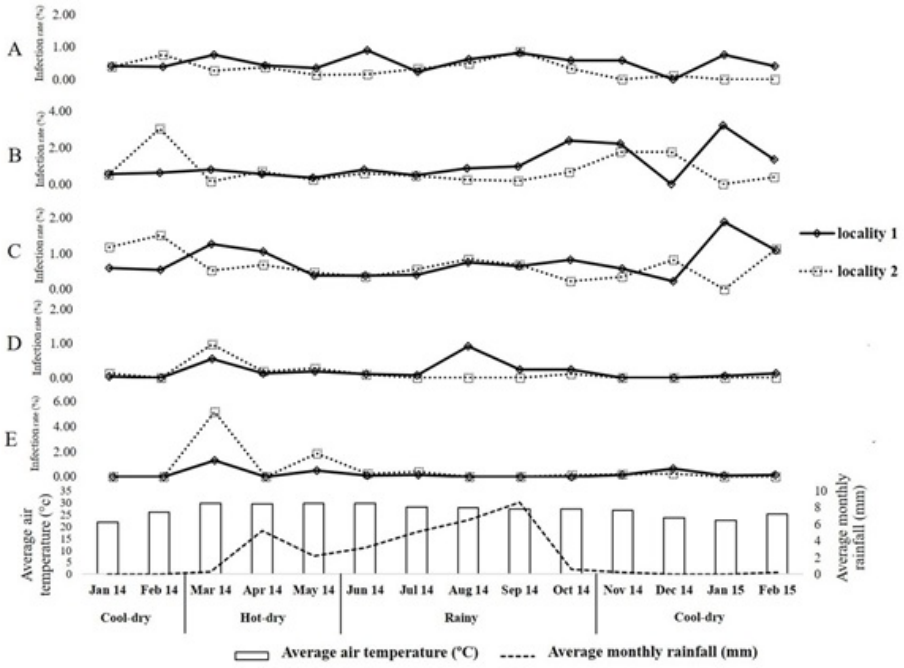

Figure 2 Variation in the pattern of mean daily temperature (°C) and mean rainfall (mm) and the prevalence of top 5 trematode infections (A = Xiphidiocercariae type virgulate 1, B = Xiphidiocercariae type virgulate 2, C = Xiphidiocercariae type virgulate 3, D = Ophthalmoxiphidiocercariae and E = Pleurolophocercous (*Opisthorchis viverrini*) in *Bithynia siamensis goniomphalos* correlated to seasons.

were examined for seasonal fluctuation such as Xiphidiocercariae type virgulate 1, Xiphidiocercariae type virgulate 2, Xiphidiocercariae type virgulate 3, Ophthalmoxiphidiocercariae, and Pleurolophocercous (*O. viverrini*) as shown in figure 2.

The prevalence of Xiphidiocercariae type 1 infection was peaked in rainy season in locality 1 and locality 2 (Figure 2A). The prevalence of Xiphidiocercariae type virgulate 2 infection was peaked in cool-dry season in locality 1 and locality 2 (Figure 2B). The prevalence of Xiphidiocercariae type virgulate 3 was peaked in hot-dry and cool-dry in locality 1 and locality 2 in cool-dry season (Figure 2C). The prevalence of Ophthalmoxiphidiocercariae infection was peaked in hot-dry and rainy season in 2 localities (Figure 2D). The prevalence of Pleurolophocercous (*O. viverrini*) infection was peaked in hot-dry season in locality 1 and locality 2 as shown in figure 2E.

Discussion

Bithynia siamensis goniomphalos snails serve as intermediate host of various trematodes, especially *Opisthorchis viverrini*, the liver fluke. This study investigated the factors that might influence trematode infection in *B. siamensis goniomphalos* snail in rice paddy fields. The top 5 dominant cercarial types were found Xiphidiocercariae type virgulate 1, Xiphidiocercariae type virgulate 2, Xiphidiocercariae type virgulate 3, Ophthalmoxiphidiocercariae, and Pleurolophocercous (*O. viverrini*).

The Xiphidiocercariae infection is the most commonly found and highest prevalence infection in *B. siamensis goniomphalos* snail similar with the previous study.[6] The Xiphidiocercariae type virgulate belong to the family Lecithodendriidae, which are parasites of prosobranch gastropods, in arthropods (insects) and fish, and in intestine of amphibians, crocodilians, lizards, snakes, birds, and mammals.[13] The Xiphidiocercaria was the highest prevalence of infection in *B. siamensis goniomphalos* snails, this was probably because of abundant diversity of intermediate host as above and available of definitive hosts in that areas.

Gender of snail host was one of factors that was influent with trematode infection. The results show that female snail was higher prevalence of infection with Pleurolophocercous (*O. viverrini*) and Ophthalmoxiphidiocercariae than male snail. On another hand, the study of *O. viverrini* infection in male and female *B. siamensis goniomphalos* snails were not different in both of fields and experiment.[14] While, there were no evidence of Ophthalmoxiphidiocercariae infection in gender snail host, the further study of this type of cercariae and *B. siamensis goniomphalos* snail would be advantaged.

Field investigation in this study reveals that 3 cercarial types including Xiphidiocercariae, Ophthalmoxiphidiocercariae and *O. viverrini* are associated with shell sized snails. Large-sized snails were higher prevalence of infection with these cercariae than medium-sized snails. These findings were in accordance with previous field study that *B. siamensis goniomphalos* snails which larger than 8 mm in shell size were higher prevalence of infection with *O. viverrini* same as previous reports.[15,16] While, experimental studies of *O. viverrini* infection in *B. siamensis goniomphalos* snails showed that small-sized snails were more susceptible for *O. viverrini* infection than larger sized snails.[17] From our results could be explained by assuming that the snails got infection since they were young and took several months for releasing free swimming cercariae when they became adult with greater sized snails.

In addition, from Odds ratio of seasonal factors and prevalence of trematode infections revealed that the snails were higher prevalence of infection with 3 types of cercariae that is Xiphidiocercariae, Pleurolophocercous (*O. viverrini*), Ophthalmoxiphidiocercariae in rainy season and cool-dry season than hot-dry season. The prevalences of Xiphidiocercariae type virgulate 1, 2, 3 infections were similary peaked which were high in cool-dry season during January-February in both localities. These findings were consistent with the study of Namsanor and colleague[7], reported that the highest prevalence of *O. viverrini* and virgulate infection were found in cool-dry season when compared with rainy and hot-dry seasons. These results could be explained that they were belong to the same family of Lecithodendriidae and the development of molluscan stage was the same time consumption. While, the prevalence of *O. viverrini* infection was peaked in rainy season (in locality 1 and 2), because of rain water flushed the contaminated eggs into water bodies in this season.

The seasonal fluctuation of prevalence of trematode infections was varied due to the consequences of many factors such as environment of locality, collection time, type of trematode,

seasons, average monthly rainfall, average daily air temperature etc. The fluctuation of prevalence of trematode infections in between 2 localities, was hypothesized that the different type of environment sampling area was affected to snail population and trematode transmission. For instance, in locality 1 and locality 2 located at the same irrigation area but locality 1 located near primary school which was available of fish pond and always found fish eating birds nearby. While locality 2 located near fresh market and found stray dogs and cats in this locality. The different of those two localities were environment and definitive hosts that would be possibly contaminated eggs of trematodes into those localities. This finding suggested that the control strategy of transmission should be considered the transmission from animal reservoir hosts especially stray dogs and cats and drug treatment for infected human host.

For physicochemical factors including the average monthly rainfall, the average daily air temperature, and the salinity was not significantly associated with prevalence of any trematode infection in both localities. Because of the localities of this study in irrigation areas where water filled in rice paddy fields from irrigation canals even dry periods. So, the rainfall and also salinity would not much impact to the prevalence of trematode infection of snails in these irrigation areas.

Conclusions

To conclude that, this study reveals that *B. siamensis goniomphalos* snails served as intermediate host of various trematodes of 14 and 11 morphologically types of cercariae were in locality 1 and locality 2 of rice paddy fields in Khon Kaen Province, respectively. The top 5 dominant cercarial types were Xiphidiocercariae type virgulate 1, Xiphidiocercariae type virgulate 2, Xiphidiocercariae type virgulate 3, Ophthalmoxiphidiocercariae, Pleurolophocercous (*O. viverrini*). Female snail was higher prevalence of infection with Pleurolophocercous (*O. viverrini*) and Ophthalmoxiphidiocercariae than male snail. Large- sized snails were higher prevalence of infection with Xiphidiocercariae, Ophthalmoxiphidiocercariae and *O. viverrini* than were medium-sized snails. The snails were higher prevalence of infection with 3 cercarial types including Xiphidiocercariae, Pleurolophocercous (*O. viverrini*), Ophthalmoxiphidiocercariae in rainy season and cool-dry season than hot-dry season. The physicochemical factors including the average monthly rainfall, the average daily air temperature, and the salinity were not significantly associated with prevalence of any trematode infection in both localities.

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