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Original Article

Cercarial Trematode Infections in Freshwater Snails of Maha Sarakham Province, Northeast Thailand

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Abstract

Background: Trematode-borne diseases remain an important public health issue in Thailand, where freshwater ecosystems support a wide diversity of snail species that act as intermediate hosts. This research aimed to determine the prevalence of parasitic infections among freshwater snails in Maha Sarakham Province.

Methods: This study employed a cross-sectional survey design to ascertain the prevalence of fluke larvae in freshwater snails in Maha Sarakham Province, Thailand during April 2023. Snail samples were selected from four freshwater bodies using the counts per unit of time methodology, with five distinct sampling points established at each site. Cercaria larvae detection was conducted using the cercaria shedding method. The cercariae were examined under a stereomicroscope to classify them based on morphological characteristics.

Results: Among the 959 individual snails examined, 11 species of freshwater snails were identified, including three cercarial trematodes, Pleurolophocercous cercaria, Furcocercous cercaria, and Xiphidiocercaria, in which *Lymnaea auricularia rubiginosa* and *Bithynia siamensis goniomphalos* were infected. The overall prevalence of trematode infection was 1.04%, with 10 infected snails. The Chi Long Forest Park Reservoir exhibited the highest infection rate at 2.33%, followed by the Nang Chong Stream at 0.60% and the Ban Koeng Reservoir at 0.45%, respectively. The prevalence of trematode infections in four freshwater bodies was not significantly different (P>0.05).

Conclusion: The findings of this study provide essential baseline data for developing effective trematode control and prevention strategies in the study area. Accordingly, targeted management of intermediate host snail populations is critical for interrupting the trematode life cycle and minimizing transmission risk.

Introduction

Trematode infections remain an important public health problem worldwide, particularly in tropical regions such as Southeast Asia. An estimated 40–50 million people are infected with foodborne intestinal trematodes, and nearly 700 million are at risk (1, 2). In this region, 59 species of foodborne intestinal flukes infect humans across several families (3). Two liver flukes, *Clonorchis sinensis* and *Opisthorchis viverrini*, account for approximately 25 million infections, with *O. viverrini* being highly endemic in Thailand and Lao PDR (2, 4). In Thailand, a 2009 national survey reported an overall liver fluke prevalence of 8.7% an estimated 6 million individuals, with the highest prevalence in the Northeast (16.6%) (5). More recent data from Maha Sarakham and Khon Kaen provinces reported *O. viverrini* infection rates of 16.0% and 19.4%, respectively (6, 7).

Human trematode infections are primarily associated with the consumption of raw or undercooked fish, snails, or other aquatic organisms that function as intermediate hosts (8, 9). Freshwater snails serve as the first intermediate hosts, supporting the development and release of cercariae that subsequently infect fish, particularly cyprinids, in which metacercariae form (10). Infection with *O. viverrini* is strongly associated with cholangiocarcinoma (11,12), while ruminant schistosomes such as *Schistosoma spindale* and *S. indicum* can also cause cercarial dermatitis and schistosomiasis in humans (13,14). Recent studies from the Bangkok Metropolitan Region found 1,275 infected snails from 12 species, with an overall prevalence of 4.3%, and identified *Bithynia siamensis siamensis* as a high-risk species due to its high cercarial prevalence and diversity (15).

Monitoring trematode infections in snails is essential for understanding parasite transmission and predicting epidemiological trends (4, 16). However, data on trematode infections among freshwater snails in Maha Sarakham Province remain limited, despite the presence of important water bodies used for consumption and daily activities. Therefore, this study aimed to determine the prevalence and diversity of trematode infections in freshwater snails from major aquatic habitats in Maha Sarakham Province, Thailand.

Materials and Methods

Study design

This study employed a cross-sectional survey design to ascertain the prevalence of fluke larvae in freshwater snails during April 2023 from natural freshwater bodies in Maha Sarakham Province (Fig. 1 and Table 1). Four bodies of freshwater were meticulously surveyed for snail populations: 1) Ban Koeng Reservoir in Ban Koeng, Koeng Subdistrict, Mueang Maha Sarakham District, 2) Huai Wang Loeng Stream, situated in Ban Hua Khua, Tha Khon Yang Subdistrict, Kantharawichai District, 3) Nang Chong Stream, also located in Ban Hua Khua, Tha Khon Yang Subdistrict, Kantharawichai District, and 4) Chi Long Forest Park Reservoir in Ban Wang Wa, Tha Khon Yang Subdistrict, Kantharawichai District.

Sampling Method

Snail samples were selected using the counts per unit of time methodology (17), with five distinct sampling points established at each site. These sampling points were strategically chosen in areas exhibiting a high concentration of snails, typically characterized by shallow waters with plant cover and aquatic vegetation. At each sampling point, five researchers collected samples for 10 min, gathering as many snails as possible. The collected snails were subsequently identified based on their morphological characteristics (18,19), cleaned, and placed in transparent plastic containers filled with chlorine-free water. The containers were securely sealed and prepared for subsequent examination.

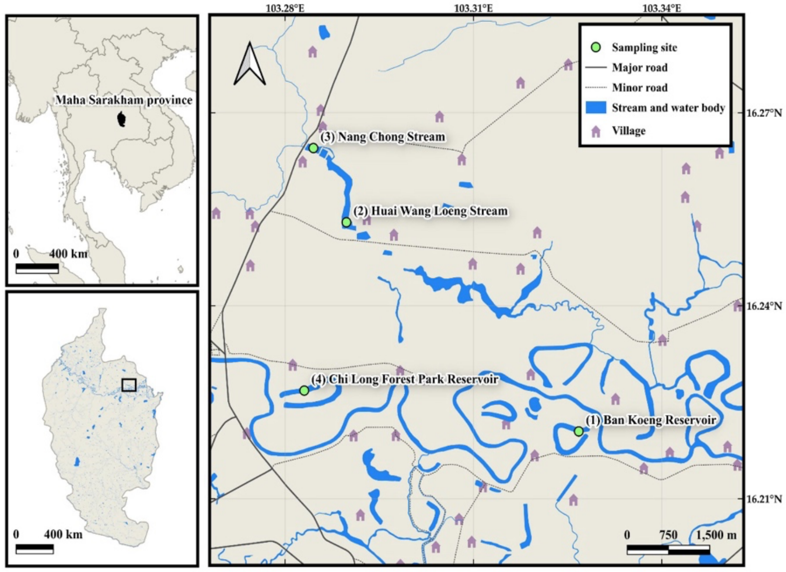

Fig. 1: Map showing the locations of snail sampling sites in Maha Sarakham Province, Thailand

Table 1: Geographic coordinates of snail sampling sites in Maha Sarakham Province, Thailand

No	Location	District	Subdistrict	Coordinates
1	Ban Koeng Reservoir	Mueang Maha Sarakham	Koeng	16°13'14.97"N/ 103°19'51.21"E
2	Huai Wang Loeng Stream	Kantharawichai	Tha Khon Yang	16°15'15.90"N/ 103°17'29.11"E
3	Nang Chong Stream	Kantharawichai	Tha Khon Yang	16°15'58.81"N/ 103°17'8.90"E
4	Chi Long Forest Park Reservoir	Kantharawichai	Tha Khon Yang	16°13'38.57"N/ 103°17'3.30"E

Method for Detecting Cercariae in Snails

Cercariae detection was conducted using the cercariae shedding method. Each snail was placed in a transparent plastic container measuring 3 cm in diameter and 2.5 cm in height, with one snail allocated per container. Each container was filled with 10 mL of chlorine-free water, and the lid, equipped with perforations for ventilation, was sealed tightly. The snails were exposed to a 40-watt yellow light for 6 h to induce cercarial shedding. The presence of cercariae was determined based on their morphology and movement observed under a stereomicroscope (20). The containers were subsequently examined under a stereomicroscope to identify the cercariae based on their morphological characteristics (21), and the size was measured in length.

Statistical analysis

Descriptive statistics were used to summarize the raw data, organized and analyzed in a Microsoft Excel spreadsheet. Snail species abundance was calculated as the proportion of individuals of each species relative to the total number of snails collected. Infection prevalence was determined by dividing the number of snails positive for cercariae by the total snail sample and expressing the result as a percentage. Differences in infection prevalence among the four sampling sites were assessed using the chi-square test. A significance level of *P*-value<0.05 was applied.

Ethics approval

This research protocol was approved by the Mahasarakham University Ethics Committee for Animal Research (IACUC-MSU-22/2022).

Results

Overall, 959 freshwater snails were collected in April 2023 from four freshwater bodies in Maha Sarakham Province: Chi Long Forest Park Reservoir, Huai Wang Loeng Stream, Nang Chong Stream, and Ban Koeng Reservoir. Eleven snail species were identified, and their shell morphology was illustrated in Fig. 2. The most abundant species was *Pomacea canaliculata* (39.0%), followed by *Filopaludina polygramma* (17.3%), *Bithynia siamensis goniomphalos* (16.8%), and *F. martensi* (16.0%) (Table 2). The Chi Long Forest Park Reservoir had the highest overall infection rate at 2.33% (7/300), with *B. s. goniomphalos* snails at 77.77% (7/9), followed by Nang Chong Stream at 0.60% (2/336), with *L. a. rubiginosa* at 100% (2/2) infection rate, and Ban Koeng Reservoir snails with an overall infection rate of 0.45% (1/223) and infected *B. s. goniomphalos* snails at 0.71% (1/140). There were no cercarial infections found in snail samples from Nang Chong Stream (Table 3).

Fig. 2: Shell morphology of 11 snail taxa collected in this study

Table 2: Prevalence of cercarial infection in freshwater snails examined from four freshwater bodies in Maha Sarakham Province, Thailand, with the numbers of examined snails, species abundance, and infected snails.

Examined snails	Overall				Pleurolophocercous cercaria		Furcocercous cercaria		Xiphidiocercaria	
	No. of examined snails	Species abundant (%)	No. of infected snails	Prevalence (%)	No. of infected snails	Prevalence (%)	No. of infected snails	Prevalence (%)	No. of infected snails	Prevalence (%)
Bithynia siamensis goniomphalos	161	16.8	8	4.97	–	–	3	1.86	5	3.11
Filopaludina martensi	153	16	0	–	–	–	–	–	–	–
Filopaludina polygramma	166	17.3	0	–	–	–	–	–	–	–
Filopaludina speciose	51	0.5	0	–	–	–	–	–	–	–

Table 2: Continued…

Indoplanorbis exustus	11	0.1	0	–	–	–	–	–	–	–
Lymnaea auricularia rubiginosa	11	0.1	2	18.18	2	18.18	–	–	–	–
Melanoides tuberculate	14	0.2	0	–	–	–	–	–	–	–
Pomacea canaliculata	374	39	0	–	–	–	–	–	–	–
Anentome helena	10	0.1	0	–	–	–	–	–	–	–
Wattebledia crosseana	3	0.3	0	–	–	–	–	–	–	–
Tarebia granifera	15	0.2	0	–	–	–	–	–	–	–
Total	959	100	10	1.04	2	0.21	3	0.31	5	0.52

Table 3: Cercariae infection rate of snails from four water bodies in Maha Sarakham Province, Thailand

Snail species	Locality / Number of infected snails (Infection rate)				No. of snails infected/ examined	Prevalence (%)
	Ban Koeng Reservoir	Huai Wang Loeng Stream	Nang Chong Stream	Chi Long Forest Park Reservoir		
Bithynia siamensis goniomphalos	1/140 (0.71%)	1	11	7/9 (77.77%)	8/161	4.97
Filopaludina martensi	65	–	44	44	0/153	0
Filopaludina polygramma	–	–	166	–	0/166	0
Filopaludina speciose	–	51	–	–	0/51	0
Indoplanorbis exustus	–	11	30	16	0/57	0
Lymnaea auricularia rubiginosa	1	7	2/2 (100%)	1	2/11	18.18
Melanoides tuberculate	–	–	13	1	0/14	0
Pomacea canaliculata	–	30	45	229	0/304	0
Anentome helena	14	–	10	–	0/24	0
Wattebledia crosseana	3	–	–	–	0/3	0
Tarebia granifera	–	–	15	–	0/15	0
Total	1/223 (0.45%)	100	2/336 (0.60%)	7/300 (2.33%)	10/959	1.04

Trematode cercariae were detected in 10 snails belonging to two species, resulting in an overall infection prevalence of 1.04% (Table 2). *Lymnaea auricularia rubiginosa* showed the highest prevalence relative to its own population (18.18%), whereas *B. s. goniomphalos* had a lower prevalence (4.97%). The greatest diversity of cercarial infections was observed in *B. s. goniomphalos*, which harbored two cercarial morphotypes—furcocercous and xiphidiocercariae—while *L. a. rubiginosa* was infected only with pleurolophocercous cercariae. No cercarial infections were detected in the remaining nine species/subspecies: *F. polygramma*, *F. martensi*, *F. speciosa*, *Indoplanorbis exustus*, *Melanoides tuberculata*, *P. canaliculata*, *Anentome helena*, *Wattebledia crosseana*, and *Tarebia granifera*. In total, three cercarial morphotypes were recorded: pleurolophocercous, furcocercous, and xiphidiocercariae (Fig. 3).

Xiphidiocercariae were the most frequently detected morphotype, occurring in five *B. s. goniomphalos* individuals from Chi Long Forest Park Reservoir (3.11% of the Bithynia population; 0.52% of the total snail population). Furcocercous cercariae were found in three Bithynia snails—one from Ban Koeng Reservoir and two from Chi Long Forest Park Reservoir—representing 1.86% of the Bithynia population (0.31% of the total population). The lowest number of infections occurred in *L. a. rubiginosa*, where two individuals from Huai Wang Loeng Stream were infected with pleurolophocercous cercariae; although the absolute number was low, the infection rate within this species was relatively high (18.18%; 0.02% of the total population). Overall, trematode infection rates did not differ significantly among the four freshwater bodies ($P>0.05$).

The cercariae were categorized based on their morphological characteristics into three groups. Pleurolophocercous cercariae were found in *L. a. rubiginosa* (2 snails) at Huai Wang Loeng. The body is oval-shaped, with attachment organs located at the head (oral sucker) and at the abdomen (ventral sucker). The tail is straight and significantly longer than the body (Fig. 3A and B). Furcocercous cercaria was identified in *B. s. goniomphalos* at Chi Long Forest Park Reservoir and Ban Koeng Reservoir. A distinguishing feature of this cercaria is the bifurcated tail, referred to as furcocercous, and the absence of a pharynx (Fig. 3C and D). Xiphidiocercaria was identified in five *B. s. goniomphalos* snails at Chi Long Forest Park Reservoir. This cercaria is characterized by its oval, unpigmented body, with an oral sucker located at the anterior end and a pharynx positioned above the digestive tract. The ventral sucker is centrally located and smaller than the oral sucker (Fig. 3E and F).

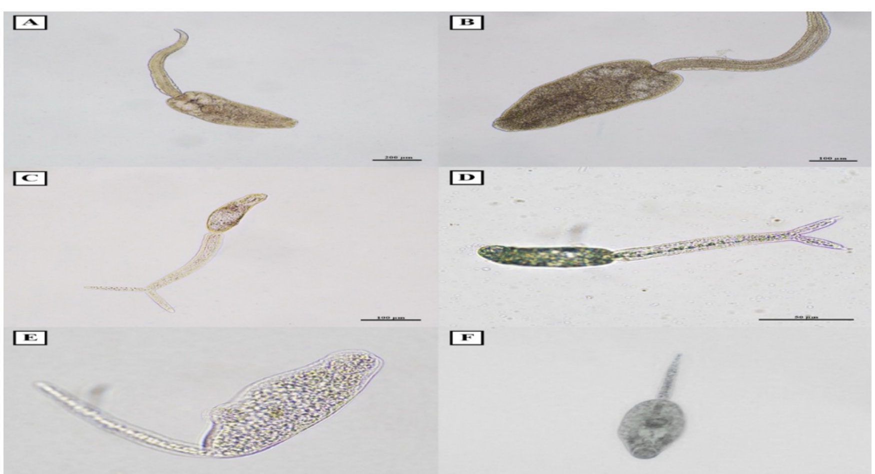

Fig. 3: Cercariae types in snails: A and B=Pleurolophocercous cercaria (in *L. a. rubiginosa*), C and D=Furcocercous cercaria (in *B. s. goniomphalos*), E and F=Xiphidiocercaria (in *B. s. goniomphalos*)

Discussion

Several studies have documented freshwater mollusk diversity and emphasized their crucial role as intermediate hosts of medically important parasites (22–31). Many of these snails participate in the life cycle of trematodes by releasing cercariae that subsequently infect cyprinoid fish as the next intermediate host (2, 5, 10). The most abundant species observed in this study, *P. canaliculata*, has previously been reported as an intermediate host of the rat lungworm, *Angiostrongylus cantonensis* (32,33). In addition, *Bithynia* spp. are well recognized as key intermediate hosts of the liver fluke *O. viverrini*, particularly *B. s. goniomphalos* (34–37). Other freshwater snails, including *Bithynia*, *Filopaludina*, and *Indoplanorbis* have also been reported to act as first or second intermediate hosts of echinostome intestinal flukes (38–40). Furthermore, schistosome cercariae (furcocercariae), which cause cercarial dermatitis, have been recovered from *M. tuberculata* in southwest Iran (41) and from *I. exustus* in southern Thailand, with the latter infection molecularly identified as *Schistosoma indicum* (14). Besides, lymnaeid snails also act as intermediate hosts of *Fasciola gigantica* in Thailand (42, 43). Collectively, several snail species present in the study area have the potential to serve as intermediate hosts of medically important parasites, underscoring their epidemiological significance.

The overall prevalence of trematode infection in freshwater snails in this study was low (1.04%; 10/959), aligning with recent reports from several provinces in Thailand, including Chiang Rai, Ubon Ratchathani, and Sakon Nakhon, where prevalences ranged from 1.39% to 1.69% (22-24). Among the four freshwater bodies surveyed, the Chi Long Forest Park Reservoir showed the highest infection rate (2.33%), followed by the Nang Chong Stream (0.60%) and the Ban Koeng Reservoir (0.45%), while no infections were detected at the Huai Wang Loeng Stream. Although infection rates varied numerically, differences among sites were not statistically significant ($P>0.05$), likely due to the low number of infected snails (n=10), which limited statistical power. Therefore, a Type II error cannot be excluded. This overall prevalence was substantially lower than that reported in other surveys conducted in Maha Sarakham Province and multiple areas in Thailand, where infection rates between 4.3% to 17.27% have been documented (25-30), as well as a large-scale survey in northern Thailand reporting an overall prevalence of 14.78% (31). Similarly, the prevalence observed in this study was markedly lower than that reported from neighboring Asian countries, including Lao PDR, Nepal, Sri Lanka, and Iran (44-46). Such pronounced spatial variation in cercarial prevalence is consistent with the ecological complexity of trematode transmission, which depends on the availability of suitable intermediate and definitive hosts, as well as local environmental conditions. Anthropogenic disturbances, including habitat modification, agricultural practices, and the introduction of non-native species, can alter snail community structure and disrupt parasite life cycles, potentially leading to reduced transmission and lower observed prevalence. These factors may partly explain the comparatively low infection rate detected in this study despite previous reports of higher prevalence in the same region. Such pronounced spatial variation in cercarial prevalence is consistent with the ecological complexity of trematode transmission, which depends on the availability of suitable intermediate and definitive hosts and on local environmental conditions (47). Anthropogenic disturbances, including habitat modification, agricultural practices, and the introduction of non-native species, can alter snail community structure and disrupt parasite life cycles, potentially leading to reduced transmission and lower observed prevalence (48-49). These factors may partly explain the comparatively low infection rate detected in this study despite previous reports of higher prevalence in the same region.

Previous studies have reported exceptionally high trematode diversity in *B. s. goniomphalos*, with up to 20 morphologically distinct cercarial types, including *O. viverrini* cercariae, identified in Thailand and Lao PDR. This high diversity has been attributed to larger sample sizes and broader geographic coverage (36). In the present study, *B. s. goniomphalos* was also found to harbor cercarial trematodes; however, only xiphidiocercariae and furcocercous cercariae were detected. These findings are consistent with recent surveys from Thailand and Lao PDR reporting variable cercarial assemblages and infection patterns in this species (25, 27, 36–37), as well as integrative studies of snail diversity and trematode infections (23–24, 28). The absence of *O. viverrini* cercariae may reflect the generally low prevalence of this parasite in its snail host reported in previous studies (50–53). In contrast, *L. a. rubiginosa* was infected exclusively with pleurolophocercous cercariae, consistent with reports from Chiang Mai Province (30). However, other studies from the same region have documented no cercarial infections in this species, indicating marked spatial and temporal variation (40). Although all *L. a. rubiginosa* specimens from Nang Chong Stream were infected in the present study, the limited sample size precludes firm conclusions, underscoring the need for further sampling to clarify the dynamics of cercarial transmission.

Conclusion

This study provides valuable baseline data on the prevalence of trematode infections in freshwater snails in Northeast Thailand. The findings enhance understanding of parasite transmission dynamics and support the development of effective control and prevention strategies for fluke-related diseases in humans. Given the continued consumption of raw or undercooked freshwater snails and fish in the region, these results are particularly relevant for reducing public health risks. Moreover, the data generated may inform future research and guide policy decisions aimed at protecting communities reliant on freshwater ecosystems. Targeted management of intermediate host snail populations is essential to disrupt the trematode life cycle and reduce transmission risk in endemic areas.

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Conflict of interest

The authors declare that there is no conflict of interest.

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