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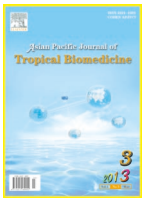

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Epidemiology of cercarial stage of trematodes in freshwater snails from Chiang Mai province, Thailand

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PEER REVIEW

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Comments

This is a good study providing important information on trematode life cycle and biology. A high overall prevalence (17.3%) of freshwater snails with trematode cercariae highlights necessity of prevention and control of zoonotic trematode infections.
(Details on Page 242)

ABSTRACT

Objective: To investigate the epidemiological situation of cercarial trematodes infection in freshwater snails from different water resources in Chiang Mai province, Thailand. **Methods:** The snail specimens were collected from 13 districts of Chiang Mai province during April 2008 to February 2012. The prevalence of cercarial infection in snails was investigated using the crushing method. The drawing was done with the help of a camera lucida for the morphological study. **Results:** A total of 2479 snail individuals were collected and classified into 7 families, 11 genera, and 14 species, Among them, 8 snails species were found to be infected with an overall prevalence of 17.27% (428/2479), which infected with nine groups of cercariae; gymnocephalous cercaria, strigea cercaria, megalurous cercaria, monostome cercaria, parapleurolophocercous cercaria (*Haplorchis cercaria*), pleurolophocercous cercaria, furcocercous cercaria (*Transversotrema cercaria*), xiphidiocercaria, and virgulate cercaria. The parapleurolophocercous cercaria was found to be the dominant type among the cercarial infection in the snails (64.25%). **Conclusions:** The various species of snails found in the research location act as the intermediate hosts for the high prevalence of parasitic infection of many species of mammals. This work will provide new information on both the distribution and first intermediate host of trematodes.

KEYWORDS

Chiang Mai, Trematodes, Cercariae, Pleurolophocercous, Prevalence, Freshwater snails

1. Introduction

The life cycle of digenetic trematodes is complicated[1]. To most species, snails act as intermediate host, in which several developing larval stages such as sporocysts, rediae and cercariae are set up[2]. Moreover, freshwater snails can have an important effect on trematodes. As they are extensively widespread in freshwater streams, the trematodes are able to produce high number of cercariae for their transmissions. The outbreaks of parasitic infection have been continually reported in Thailand, especially in Chiang Mai province, Thailand. This area is covered by diverse ecosystems of farm forestry, along with plenty of water resources, producing a high biodiversity to support the life cycle of trematodes for the infection of both humans and animals. Epidemiological surveys throughout 2008-2011 have also shown an increase of trematode infections in this area[3-7].

In Thailand, many species of freshwater snails were reported to be intermediate hosts. For example, *M. tuberculata*, *Melanoides jujicostis* and *Neoradina prasongi* are the intermediate hosts of *Haplorchis taichui* and *Haplorchis pumilio*[8]. Other snail species, *Filopaludina* spp. and *Lymnaea* spp. were reported to act as the first and second most common intermediate hosts of trematodes in the family Echinostomatidae[9]. Furthermore, the planorbis snails are intermediate hosts of blood fluke in family Schistosomatidae[10,11].

However, only a few studies have been concerned

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with the cercaria infectious potential in northern Thailand. For instance, 4 types of cercariae (virgalate cercaria, lophocercous cercaria, monostome cercaria and pleurolophocercous cercaria) were discovered in *Bithynia funiculata* with an overall prevalence of 9.60%[12]. Moreover, 2 types of cercariae (parapleurolophocercous cercaria and xiphidiocercaria) were found in thiarid snails from the Khek river, Phitsanulok province with an overall prevalence of 0.90%[13]. However, only a few snail species were investigated. Information on the species composition of cercariae is a pre-requisite for any study on the epidemiology of the veterinary or biomedical importance of trematodes. Hence, a study of the epidemiological situation of cercarial infection in freshwater snails in Chiang Mai province was carried out, basing on the original data from a large scale survey of cercaria infections. This is the first step for providing new information on the latest distribution of trematode infection and for the development of the effective control measures in Chiang Mai.

2. Materials and methods

2.1. Study sites

The study sites were located in 13 districts of Chiang Mai province. They were selected on areas of public health problems with trematode infections. The coordinates for each sites were recorded using the global positioning system. The specific coordinates are as follows: Chiang Dao (47Q 0496661 UTM 2143175), Chaiprakarn (47Q 0514443 UTM 2200541), Chom Thong (47Q 0489656 UTM2051812), Doi Lo (47Q 0486618 UTM 2048540), Doi Saket (47Q 0513597 UTM 2091766), Fang (47Q 0510985 UTM 2206053), Hang Dong (47Q 0492365 UTM 2066951), Mae Ai (47Q 0530078 UTM 2200561), Mae Rim (47Q 0496661 UTM 2143175), Mae Taeng (47Q 0507670 UTM 2113464), Muang Chiang Mai (47Q 0494731 UTM 2079399), San Pa Tong (47Q 0486557 UTM 2051801) and Saraphi (47Q 0504652 UTM 2068211) (Figure 1).

2.2. Snail specimens

The snail specimens were collected by hand during April 2008 to February 2012. After collection, the snails were identified using the methods of Brandt (1974)[14], and then they were crushed to examine under light microscope to investigate cercarial infection. The alive cercariae were vitally stained with 0.5% neutral red and identified according to morphology described by Schell and Ito[15,16]. In addition, the cercarial specimens were stained with Delafield's haematoxylin or borax carmine, dehydrated in an alcohol series, cleared with xylene, and mounted in permount. With camera lucida, illustrations were made to record information for a morphological characteristic study. The cercariae were identified at the family level and in some cases, the identification of the genus was possible.

Figure 1. Map of the 13 collecting sites of Chiang Mai province (●) where samples of snails were examined.

3.Results

A total number of 2 779 snail individuals were collected in Chiang Mai province, Thailand. They were classified into 7 families, 11 genera and 14 species, including *Bithynia siamensis* (*B. siamensis*), *Brotia crostula* (*B. crostula*), *Brotia wycoffi*, *Clea helena*, Eyriesia eyriesi (*E. eyriesi*), *Filopaludina dorliaris*, *Filopaludina sumatrensis polygramma*, *Filopaludina martensi*, *Indoplanorbis exustus* (*I. exustus*), *Lymnaea auricularia rubiginosa*, *Melanoides tuberculata* (*M. tuberculata*), *Pila polita*, *Tarebia granifera* (*T. granifera*) and *Thiara scabra* (*T. scabra*) (Figure 2). The results showed that, the overall prevalence of cercarial infection was 17.27% (428/2 457) in 8 snail species (Table 1).

A total of 9 groups of cercaria were found. They were divided into separate groups based on morphologically distinguishable differences according to Schell (internal organ arrangement, place and number of sucker *etc*.). They were subsequently described as follows.

Table 1
Composition of the snail species and number of cercarial infection.

Snail species		Number examined	Types of cercariae								
			Gym	Meg	Mon	Par	Ple	Str	Tra	Xip	Vir
Family Ampullariidae	*Pila polita*	105	–	–	–	–	–	–	–	–	–
Family Bithyniidae	*B. siamensis*	365	2	–	10	6	1	2	–	9	10
Family Buccinidae	*Clea helena*	76	–	–	–	–	–	–	–	–	–
Family Bulinidae	*I. exustus*	30	–	–	–	–	–	1	–	–	–
Family Lymnaeidae	*Lymnaea auricularia rubiginosa*	60	–	–	–	–	3	–	–	–	–
Family Thiaridae	*B. crostula crostula*	8	–	–	–	–	1	–	–	–	–
	Brotia wycoffi	10	–	–	–	–	–	–	–	–	–
	M. tuberculata	598	–	17	–	114	29	–	6	19	–
	T. granifera	540	–	–	3	149	15	–	–	23	–
	T. scabra	93	–	–	–	4	1	–	1	–	–
Family Viviparidae	*E. eyriesi*	90	–	–	–	2	–	–	–	–	–
	Filopaludina dorliaris	121	–	–	–	–	–	–	–	–	–
	Filopaludina sumatrensis polygramma	90	–	–	–	–	–	–	–	–	–
	Filopaludina martensi	293	–	–	–	–	–	–	–	–	–
Total		2479	2	17	13	275	50	3	7	51	10

Gym: Gymnocephalous cercaria; Meg: Megarulous cercaria; Mon: Monostome cercaria; Par: Parapleurolophocercous cercaria; Ple: Pleurolophocercous cercaria; Str: Strigea cercaria; Tra: Transversotrema cercaria; Xip: xiphidiocercaria cercaria; Vir: Virgulate cercaria.

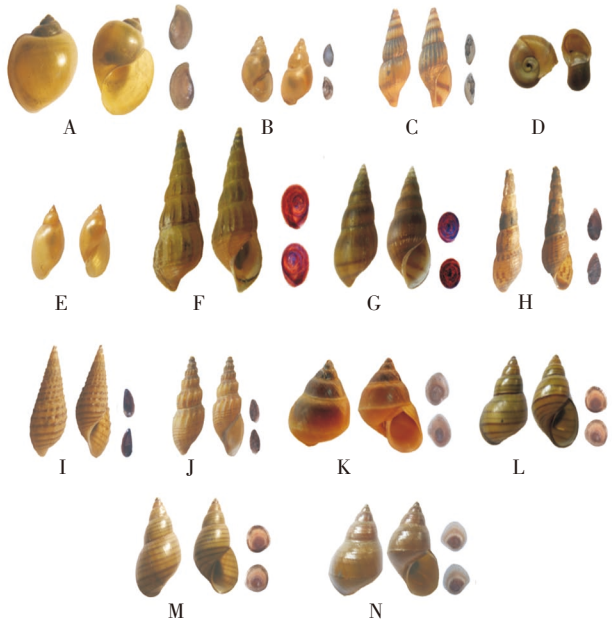

Figure 2. Shell and operculate characteristic of snails collected from the collecting sites.
A: *Pila polita;* B: *B. siamensis;* C: *Clea helena;* D: *I. exustus;* E: *Lymnaea auricularia rubiginosa;* F: *B. crostula crostula;* G: *Brotia wycoffi;* H: *M. tuberculata;* I: *T. granifera;* J: *T. scabra;* K: *E. eyriesi;* L: *Filopaludina dorliaris;* M: *Filopaludina sumatrensis polygramma;* N: *Filopaludina martensi.*

3.1. Gymnocephalous cercaria (Fasciolidae)

Snail host: *B. siamensis*

The cercaria is ovoid shaped. Oral sucker sub-terminal and short prepharynx. Esophagus bifurcates postero-lateral to ventral sucker and extended close to the posterior quarter of the body. The excretory vesicle is spherically-shaped. The main collecting duct ascends from anteroleteral wall of the vesicle, extending from the area of the pharynx and oral sucker. The excretory ducts contains a large granule. Ventral sucker, which is extremely protractible and larger than the oral sucker and, tends to be slightly posterior from the middle of its body. The tail is strongly developed and its length is almost as long as the body with dorso-ventral finfold (Figure 3A).

3.2. Furcocercous cercaria (Transversotrema cercaria)

Snail hosts: *M. tuberculata* and *T. scabra*

Cercaria body is short, flat, and dish like shaped with a yellowish-brown pigment. The body surface is covered with scale-like spines. Its pharynx is very large, while the esophagus is short and narrow. A pair of eyespots is spherically large and located in the posterior position of the pharynx. An oral sucker is absent while a ventral sucker is present medially on its body. Testes are symmetrical and within the intestinal ring and the genital pore, and are located medially. The tail is longer than the body, and with arm-like processes at the anterior end of the tail-stem (Figure 3B).

3.3. Megalurous cercaria (Philophthalmidae)

Snail host: *M. tuberculata*

The body is elongated with yellowish-brown granules. The width of the body is as the one-third from the body length. The body surface is covered with longitudinal and fine traverse wrinkles. Minute spines also cover the surface of the posterior half of the body. The sub-terminal oral sucker has the complex muscular

apparatus. The bifurcated esophagus is located in the middle of the pharynx and ventral sucker, and the intestines ended blindly near the posterior end of body. The ventral sucker is slightly larger than the oral sucker and is, located medially on its body. The tail is long as the body, elastic and slender. The tip of the long tail contains numerous adhesive gland cells and lacks an excretory canal at its tail base. Within an hour, these cercariae encyst on the surface of the bottom of the container (Figure 3C).

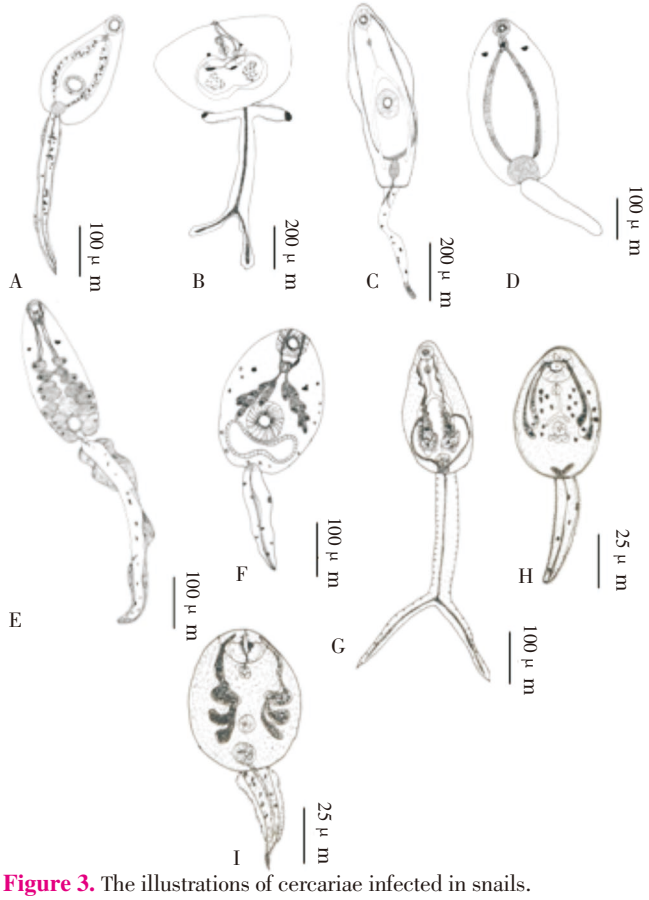

Figure 3. The illustrations of cercariae infected in snails.
A: Gymnocephalous cercaria; B: Transversotrema cercaria; C: Megalurous cercaria; D: Monostome cercaria; E: Haplorchis cercaria; F: Pleurolophocercous cercaria; G: Strigea cercaria; H: Xiphidiocercaria; I: Virgulate cercaria.

3.4. Monostome cercaria (Notocotylidae)

Snail hosts: *B. siamensis* and *T. granifera*

The cercaria body is in contractile and oval when being relax. The whole body is transparent and the anterior quarter is deeply pigmented, particularly around the eyespots. The sub-terminal oral sucker is conspicuous while the ventral sucker is absent. There is a pair of black-pigmented eyespots in the front of the triangle in the anterior dorsal area of the mature cercaria (Figure 3D).

3.5. Parapleurolophocercous cercaria (Haplorchis cercaria)

Snail hosts: *B. siamensis*, *E. eyriesi*, *M. tuberculata*, *T. granifera*, and *Thiara. scabra*

The cercaria body is in pear-shaped, which is entirely covered with minute spines. Circular oral sucker is sub-terminal and the ventral sucker is lacking. The small pharynx lies just behind the oral sucker. Two eyespots are square in shape and located on each side below the pharynx. Seven pairs of penetration glands are located between the pharynx and the posterior part of body, mainly surrounding the genital premordium and arranged in two columns with the ventral sucker. Excretory vesicles are elongated. The long tail is attached to the dorsal end of the body, with lateral finfolds nearby and dorso-ventral finfolds extending along the posterior two thirds (Figure 3E).

3.6. Pleurolophocercous cercariae (Heterophyidae)

Snail hosts: *B. siamensis*, *B. crostula*, *Lymnaea auricularia rubiginosa*, *M. tuberculata*, *T. granifera* and *T. scabra*

The cercaria is ovoid shaped. The entire body is covered with minute spines. The oral sucker is sub-terminal. A pair of rectangular conspicuous eyespots is found at the end of the anterior third of the body. The pharynx lies just behind the oral sucker. Intestinal ceaca bifurcate and extend posterior to the excretory vesicle level. Seven pairs of penetration glands are arranged in 2 bundles and are anterolateral to the genital primordial which is a relatively large triangular mass of cells that is located just anterior to the excretory vesicle. The cytogenous glands are numerous and lie on the later site of the body. The tail has almost the same body length, is slender and usually attached to the dorso-ventral finfolds. A brownish pigment is dispersed throughout the body except in the oral sucker (Figure 3F).

3.7. Strigea cercaria (Strigeidae)

Snail hosts: *B. siamensis* and *I. exustus*

The body is ovoid and its surface is covered with minute spines and is shorter than the tail stem. Furcae is long-shape with a dorsal and ventral finfold. The pharynx is very small and circular. The esophagus bifurcates the ventral sucker. Genital primordial is spherical. Two pairs of penetration glands are locates between the ceaca posterior to ventral sucker (Figure 3G).

3.8. Xiphidiocercaria (Plagiorchidea)

Snail hosts: *B. siamensis*, *M. tuberculata* and *T. granifera*

The cercaria is elongated, oval-shaped, colorless and spinose. The oral sucker is circular and its stylet is inserted into the roof of the oral sucker. The ventral sucker is post-equatorial, rounded and smaller than the oral sucker. Pre pharynx is very short. Genital primordial is globular. The tail is slender (Figure 3H).

3.9. Vergulate cercaria (Lecithodendriidae)

Snail host: *B. siamensis*

The cercaria is small in size with a prominent stylet

developing in sporocysts. The oral sucker is in the back, close to the pharynx. The esophagus bifurcates into the anterior part of the ventral sucker and encircles the intestinal caeca. The ventral sucker is terminated postacetabularly. The ventral sucker is smaller than the oral sucker. The virgulate organ is butterfly-shaped and, situated below the stylet. Additionally, here are 3 pairs of penetration glands and two anterior pairs with fine granules. A posterior pair with coarser granules is positioned medially on the body. The tail is shorter than its body (Figure 3I).

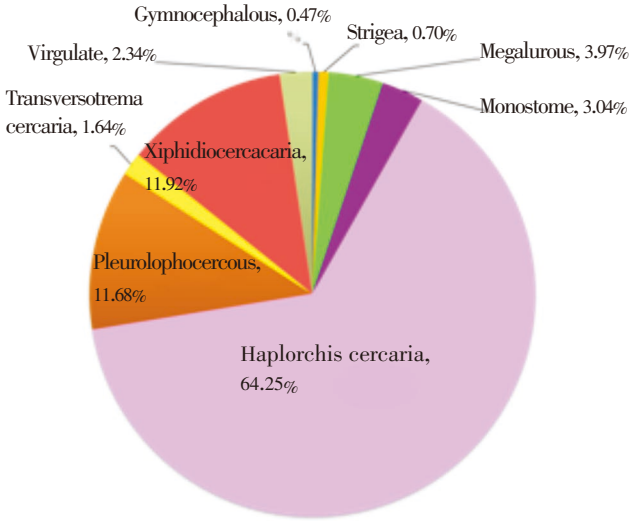

Figure 4. The proportion of cercarial infected in snails.

As shown in Figure 4, The parapleurolophocercous cercariae (Haplorchis sp.) is the dominant cercarial type infecting snails (64.25%, 275/428), while other cercaria (xiphidiocercaria, pleurolophocercous cercaria, megalurous cercaria, monostome cercaria, virgulate cercaria, Transversotrema cercaria and strigea cercaria) infected snails at a rate of only 11.92%, 11.68%, 3.97%, 3.04%, 2.34%, 1.64% and 0.70%, respectively.

4. Discussion

This study revealed a high variety of freshwater snails in Chiang Mai province. Eight of the 15 snail species serve as the intermediates host of trematode, namely *B. siamensis*, *B. crostula*, *E. eyriesi*, *I. exustus*, *Lymnaea auricularia rubiginosa*, *M. tuberculata*, *T. granifera* and *T. scabra*.

Total prevalence of cercarial infections found in this study (17.27%) was higher than that in the previous report. Mard- arhin N *et al.* revealed the prevalence of only 6.20% cercariae infection in freshwater snails form five provinces in the Northern Thailand[17]. Ngern-klun *et al.* discovered virgalate cercaria, lophocercous cercaria, monostome cercaria and pleurolophocercous cercaria in Bithynia funiculata with an overall prevalence of 9.60%[12]. This conclusion is also supported by the peculiar ecological conditions of the water resources in Chiang Mai province. It represents a complex and diverse freshwater ecosystem. The suitable conditions make cercariae able to develop in snails and fish which lead to perpetuation in their life cycle. Moreover, the generic composition of the obtained trematode larvae in the studied snails might be influenced by the anthropogenic factors and specific fauna in the observed regions, including microorganisms that could be the potential secondary or/and intermediate hosts of the larvae found in freshwater snails.

From the result, the snail in Family Thiaridae had shown a high susceptibility for parapleurolophocercous cercaria and pleurolophocercous cercaria infection. These types of cercarial infected many species of snails. It means that, there exists a very low specificity for infection in snail intermediate hosts. *Haplorchis taichui*, *Haplorchis pumilio*, *Stellantchasmus falcatus*, *Centrocestus caninus* and *Procerovum* sp. were reported to be as the cercarial stage of the intestinal trematode in family Heterophyidae[18-25]. The identification of the morphology of cercariae were similar to those described by previous reported[8,16,20]. In regard to this connection, previous surveys revealed that most metacercariae found in Mae Taeng district, Chiang Mai province belonged to the family Heterophyidae[3,6,18]. This can be considered significant evidence to support the success of this study in the identification and classification of the epidemiological situation of larval trematodes in their intermediate hosts.

Form the results, the 17 *M. tuberculata* were infected by megarulous cercariae, which have been morphologically characterized as belonging to the genus Philophtalmus. Because, these cercariae have neither a collar-like thickening nor a crown of spines, they encyst on the surface of the bottom of the container. The metacercarial cysts are flask-shaped[26]. The snail intermediate host of these cercaria was similar to those reported in the same snail species[26-29], while some were only found in Semisulcospira libertine in some study[29]. Concerning the snail hosts, *B. siamensis* were shown to be the suitable intermediate hosts for harboring a wide spectrum of cercarial infection, where gymnocephalus cercariae and virgulate cercaria were found to have infected only in one snail species. Thus, it seems that these types of cercariae have a high specificity for infection in snail hosts.

In the present study, xiphidiocercaria was discovered in the 3 snail species, *B. siamensis*, *M. tuberculata* and *T. granifera* while, previous reported found xiphidiocercaria to be present in *Lymnaea caillaudi*, *Cleopatra bulinoides*, *Lonistes carnatus*, *Thiara tuberculata* and *T. granifera*, and they were reported to be present as the cercarial stage of the trematodes in

Superfamily Plagiorchioidea[13,30,31].

In addition, for the first time, the present study discovered the strigea cercaria infecting in 2 snail species including *B. siamensis* and *I. exustus* from Chiang Mai province. Previous reports found the same type of cercaria in *B. siamensis* from 4 districts of Chiang Mai province[12].

This present work describes the Transversotrema cercaria, which was discovered from *M. tuberculata* and *T. scabra*. The morphological characteristic of this cercaria from the same and other snail species was described in the literature. Transversotrema cercaria was found in *M. tuberculata*[18] while it was found in *Melanoides terebra* and *Thiara riguettii* from several study areas in another study[32]. The viviparid and plumonate snails (*Lymnaea* spp. and *Filopaludina* spp.) were not infected by any cercariae, which is a snail species that has been commonly infected by echinostome cercaria and furcocercous cercaria. In Phang-nga province, Thailand, echinostome cercaria was found to have infected the same snail species as *Lymnaea peregra*, *Lymnaea stagnalis* and *Lymnaea. natalensis*[33-35]. This loss of this cercaria could correspond to the changes in the abundance of intermediate and definitive hosts (snails and fishes), which serve as the secondary intermediate host that affect the life cycle completion.

Therefore, this report can conclude that the freshwater snails can be considered and important determinant in monitoring the biomedical and veterinary public health. About 100 species of snails were reported to act as intermediate hosts for the trematode parasite, particularly in the family Thiaridae which harbor the larvae of the intestinal and eye flukes[26]. The species identification will be done for accuracy control in this area. The further study is will apply the experimental infection data for adults. However, the modern methods of technology used for the detection and identification process should be applied in the future research. The further study would be able to detect the species of trematodes by the PCR method in the larva and adult stages.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Freshwater snails are an important element in the life cycle of trematode parasites. Around Chiang Mai Province, Thailand, a plenty of trematode species, including *Haplorchis taichui*, *Haplorchis pumilio*, and echinostome species, have been described. However, studies on cercarial infections in freshwater snails have been scarce. Surveys on cercarial infection status in snails are expected to provide important information on ecology and host-parasite relationships of trematode infections.

Research frontiers

Nine groups of cercariae have been detected in this study, and the overall infection rate of the snails was 17.3%. This rate is higher than the figures previously reported in Chiang Mai area. However, molecular methods in detecting infected snails will be necessary in future studies. Strigeid cercariae were detected for the first time in 2 snail species in this area.

Related Reports

Mard-arhin *et al*. (2001) reported 6.2% overall cercarial positive rate in five provinces in northern, Thailand. In this study, the rate was higher, 17.3% in Chiang Mai Province.

Innovations and breakthroughs

The results reported in this report provided important information on the ecology and host-parasite relationships of various trematode species distributing in Chiang Mai Province, Thailand. This study also provided baseline information for further studies on cercarial infections using molecular techniques.

Applications

The results will be applicable to further studies on biology and epidemiology of each trematode species. The results will also be applicable to further studies on infection of each freshwater snail species with trematode cercariae. This study will be applicable for prevention and control of zoonotic trematode infections of high public health significance.

Peer review

This is a good study providing important information on trematode life cycle and biology. A high overall prevalence (17.3%) of freshwater snails with trematode cercariae highlights necessity of prevention and control of zoonotic trematode infections.

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