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Prevalence of Cercarial Infection in Freshwater Snails from Phra Nakhon Si Ayutthaya Province, Thailand

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ABSTRACT

The prevalence of cercarial infection was investigated in various species of freshwater snails from 29 sampling sites in Phra Nakhon Si Ayutthaya province, Thailand. A total of 2,210 snail specimens were classified into 11 species: *Adamietta housei*, *Bithynia siamensis, Clea helena, Filopaludina martensi, Filopaludina polygramma, Indoplanorbis exustus*, *Lymnaea auricularia*, *Melanoides tuberculata*, *Tarebia granifera*, *Thiara scabra* and *Wattebledia* sp. The overall prevalence of infection was 1.99% (44 individuals), with the highest prevalence in xiphidiocercaria (0.81%), followed by cercaria (0.37%), echinostome cercaria (0.27%), monostome cercaria (0.27%), parapleurolophocercous cercaria (0.14%), furcocercous cercaria (0.09%) and megalurous cercaria (0.05%). Furthermore, the snails in Family Thiaridae showed a high susceptibility for parapleurolophocercous cercaria. Thus, this study has provided information concerning the epidemiological situation of cercarial infection in freshwater snails, an intermediate host of trematodes. This information may be used for planning and evaluating programs for the effective control of snail – borne disease.

INTRODUCTION

Freshwater snails are important as a primary intermediate host of all trematodes that cause several snail-borne diseases in human and various economically important animal species [1,2]. For example, *Melanoides tuberculata* and *Tarebia granifera* are intermediate hosts of several blood and intestinal trematodes [3,4]. Hence, snail - borne diseases may be distributed across water resources such as irrigation canals, agricultural areas, reservoirs, rivers, and streams. These diseases have a detrimental effect on public health and socio-economic factors, made worse by the lack of an effective surveillance system and health education.

Phra Nakhon Si Ayutthaya province is situated on Chao Phraya's floodplain, Thailand. This region contains several different kinds of water resources, including rice paddies, rivers, canals, and dams, which are suitable for agriculture and fishing. This condition also promotes the abundance of intermediate hosts leading to wide dispersal of parasites within the area.

Previous investigations on the prevalence of cercarial infection in freshwater snails from central Thailand revealed that several species were intermediate hosts of important parasites. For example, *M. tuberculata* is an intermediate host of Heterophyidae (parapleurolophocercous cercaria) and Philophthalmidae (megarulous cercaria), *Indoplanorbis exustus* and *Lymnaea auricularia* are intermediate hosts of Echinostomatidae (echinostome cercaria), *Bithynia siamensis* is an intermediate host of Strigeidae (furcocercous cercaria) and Lecithodendriidae (xiphidiocercaria), and *T. granifera* is an intermediate host of Philophthalmidae (megarulous cercaria) [3-5]. Moreover, *B. siamensis*, a species commonly found in ponds, shallow reservoirs and wetlands, including rice fields and small canals, have been reported to be an intermediate host for Opisthorchiidae in the northeast of Thailand [4]. This finding was in concordance with other studies of bithynid snails: *B. fuchsiana*, *B. goniomphalos*, *B. misella*, *B. longicornis*, and *Wattebledia crosseana* in Vietnam [6]. Furthermore, there have been reports of metacercariae in fermented fish dishes from five provinces: Si Sa Ket, Sakon Nakhon, Mukdahan, Khon Kaen and Udon Thani [7]. A study of cercarial infections in freshwater snails collected from water reservoirs in Chiang Mai showed heavy infection with *Haplorchis taichui* and *Haplorchoides* sp. [8]. The prevalence of monostome cercaria were highest in *B. siamensis* from rice fields, while in *M. tuberculata*, *T. granifer*a and *T. scabra* were found to have pleurolophocercous cercaria infection [9, 10].

The purpose of this study is to investigate the epidemiological situation based on the prevalence of cercarial infection in snails from Phra Nakhon Si Ayutthaya provinces, Thailand. This investigation is

intended to gain current epidemiological position data for this area.

METHODOLOGY

Animal Ethics

All experimental procedures involving animals were conducted in accordance with the National Research Council of Thailand and approved by the Biological Experiment Animals Committee, Department of Biology, Faculty of Science, Srinakharinwirot University.

Sampling sites

The specimens were collected from 29 sampling sites in Phra Nakhon Si Ayutthaya province during August to December, 2016. Each sampling site was selected based on areas with public health problems with trematode infections using stratified random sampling method [11].

The geographical coordinates for each collecting site was recorded using the global positioning system with UTM unit. (Table 1, Figure 1).

Snail classification and cercarial examinations

Fifty individuals of each snail species from each sampling site were collected by hand and identified following the methods of Brandt (1974) [12, 13]. Each snail was examined using the method of crushing and investigating for cercarial infection under a light microscope. After that, the living cercarial specimens were stained with neutral red and classified according to morphology as described by Schell and Ito [14, 15]. Then, the cercarial specimens were stained with Aceto-orcein or haematoxylin, dehydrated using an alcohol gradient, clarified with xylene and mounted in permount. Finally, the cercariae were identified to the Family level using a camera lucida to record information on the morphological characteristics.

Table 1 The snail collection area in the Phra Nakhon Si Ayutthaya province, Thailand.

Sampling sites	Geographical coordinates
Tha Ruea 1	47Q685845 UTM1610825
Tha Ruea 2	47Q685195 UTM1608651
Tha Ruea 3	47Q683553 UTM1606843
Nakhon Luang 1	47Q672455 UTM1594886
Nakhon Luang 2	47Q673154 UTM1597293
Nakhon Luang 3	47Q676325 UTM1599293
Phachi 1	47Q687757 UTM1599254
Phachi 2	47Q681016 UTM1598098
Uthai 1	47Q681364 UTM1589385
Uthai 2	47Q680405 UTM1591288
Phra Nakhon Si Ayuttha 1	47Q667798 UTM1586333
Phra Nakhon Si Ayuttha 2	47Q664856 UTM1586748
Phra Nakhon Si Ayuttha 3	47Q664911 UTM1591194
Phak Hai 1	47Q651166 UTM1603720
Phak Hai 2	47Q636923 UTM1599604
Phak Hai 3	47Q647498 UTM1599130
Bang Pa-in 1	47Q674436 UTM1567335
Bang Pa-in 2	47Q672056 UTM1571034
Bang Pa-in 3	47Q668432 UTM1567433
Bang Pahan 1	47Q667074 UTM1608584
Bang Pahan 2	47Q666817 UTM1605093
Bang Pahan 3	47Q655554 UTM1593392
Maha Rat 1	47Q665534 UTM1608584
Ban Phraek 1	47Q667784 UTM1618144
Ban Phraek 2	47Q669935 UTM1619808
Wang Noi 1	47Q680405 UTM1591288
Wang Noi 2	47Q682021 UTM1572246
Bang Sai 1	47Q664458 UTM1564847
Bang Sai 2	47Q665056 UTM1563035

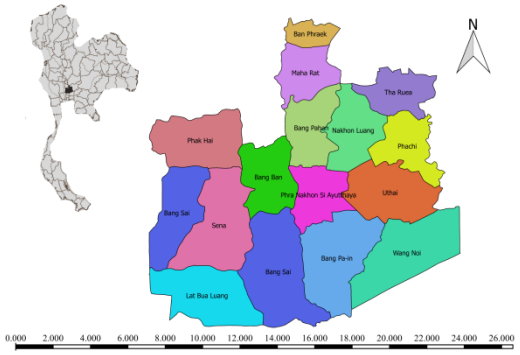

Figure 1 Map of Phra Nakhon Si Ayutthaya province.

RESULTS AND DISCUSSION

A total of 2,210 snail specimens were collected from 29 sites across Phra Nakhon Si Ayutthaya province, Thailand. Specimens were classified into 6 families comprising, 11 species as follows: *Adamietta housei*, *Bithynia siamensis, Clea helena, Filopaludina martensi, Filopaludina polygramma, Indoplanorbis exustus*, *Lymnaea auricularia*, *Melanoides tuberculata*, *Tarebia granifera*, *Thiara scabra* and *Wattebledia* sp. Regarding the cercarial infection, the overall prevalence of infection was 1.99% (44/2,210) in 11 snail species (Table 2). This result was similar to the previous report in which the prevalence of infection in freshwater snails from Chao-Phraya Basin, Central Thailand was 5.90% [3]. In addition, 7 types of cercarial stage, based on morphological characteristic appearance, were reported, including cercaria, megalurous cercaria, monostome cercaria, parapleurolophocercous cercaria and xiphidiocercaria.

The criteria for cercarial classification are internal organ arrangement, position and numbers of adhesive organ. All of the cercaria types were described as follows:

Cercariae

Intermediate hosts: *B. siamensis* and *F. polygramma*

Morphological characteristic: Large size and slender shaped with a yellowish-brown pigment. The oral sucker is located at the sub-terminal of the anterior part of the body, while the ventral sucker is present at the mid-body and its size is similar to the oral sucker. The pharynx is large with bifurcated intestine. This cercarial type does not have a tail at the posterior end of the body (Figure 2A).

Echinostome cercaria

Intermediate hosts: *B. siamensis* and *F. polygramma*

Morphological characteristic: The body is elongated with a white pigment. The oral sucker is located at the sub-terminal of the body and

Table 2 The numbers of snail specimens and number of cercarial infection.

Snail taxa		Number of snails	Types of cercariae						
			Cer	Ech	Fur	Meg	Mon	Par	Xip
Family Bithyniidae	*B. siamensis*	850	7	2	-	-	2	-	15
	Wattebledia sp.	100	-	-	-	-	4	-	-
Family Buccinidae	*C. helena*	57	-	-	-	-	-	-	-
Family Bulinidae	*I. exustus*	225	-	-	-	-	-	-	-
Family Lymnaeidae	*L. auricularia*	178	-	-	1	-	-	-	3
Family Thiaridae	*A. housei*	80	-	-	-	-	-	-	-
	M. tuberculata	143	-	-	-	-	-	2	-
	T. granifera	150	-	-	-	-	-	1	-
	T. scabra	71	-	-	-	-	-	-	-
Family Viviparidae	*F. martensi*	195	-	-	1	-	-	-	-
	F. polygramma	161	1	4	-	1	-	-	-
Total		2,210	8	6	2	1	6	3	18

Cer: Cercariae, Ech: Echinostome cercaria, Fur: Furcocercous cercaria, Meg: Megalurous cercaria, Mon: Monostome

surrounded by a collar spine. The ventral sucker presents at the mid-ventral part of the body. The esophagus is bifurcated and is located between the pharynx and ventral sucker. The cytogenous glands are numerous. The tail is flexible, slender and of similar length as the body (Figure 2B).

Furcocercous cercaria

Intermediate hosts: *L. auricularia* and *F. martensi*

Morphological characteristic: The body of this cercarial type is flat and long shaped, and is unpigmented. The oral sucker is small and located at the terminal part of the body. The ventral sucker presents at about 2/3 of the body. The intestine is long, narrow, and bifurcated. There are eye spots with spherical shape, located at the anterior and there are two pairs of penetration glands. The tail is longifurcate, divided into bifurca and longer than the body (Figure 2C).

Megalurous cercaria

Intermediate hosts: *F. polygramma*

Morphological characteristic: The body is elongated and has white pigment with numerous granules. The oral sucker is located at the sub-terminal of the body. The ventral sucker is present at the middle of the body and larger than the oral sucker. The esophagus is Y-shaped. The tail is similar to the body in length and slender. There are adhesive gland cells located at the tip of long tail (Figure 2D).

Monostome cercaria

Intermediate hosts: *B. siamensis* and *Wattebledia* sp.

Morphological characteristic: The body is ovoid shaped. All parts of the body are transparent. The oral sucker is globular in shape and located at the sub-terminal of the body. The ventral sucker is absent. The esophagus is bifurcate with dark-brown pigment at its anterior. There are eye spots, round shaped and located at the lateral of the pharynx. The tail is shorter than the body (Figure 2E).

Parapleurolophocercous cercaria

Intermediate hosts: *M. tuberculata* and *T. granifera*.

Morphological characteristic: The body is oval, pear-shaped with orange-yellow pigment and is covered with minute spines.

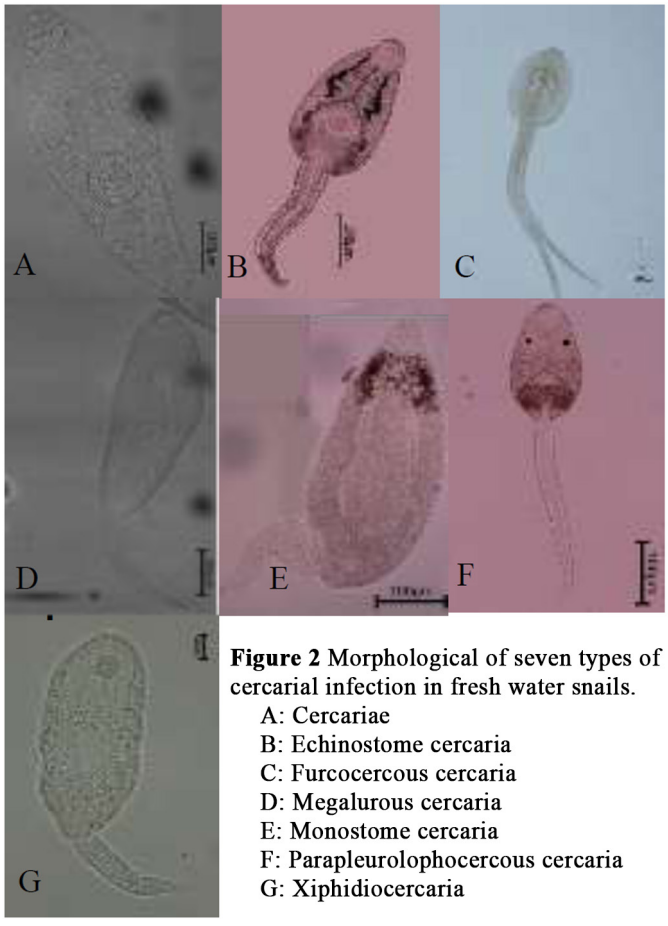

Figure 2 Morphological of seven types of cercarial infection in fresh water snails.
A: Cercariae
B: Echinostome cercaria
C: Furcocercous cercaria
D: Megalurous cercaria
E: Monostome cercaria
F: Parapleurolophocercous cercaria
G: Xiphidiocercaria

The Oral sucker is orbicular in shape and is located at the sub-terminal of the body. The ventral sucker is smaller than the oral sucker and is present near the posterior of the body. The intestine is bifurcated. One pair of eye spots, with quadrate shape, are located at the lateral of the pharynx. There are seven pairs of penetration glands arranged in 2 columns. The tail is slender and longer than the body with lateral fin folds nearby and a dorso-ventral fin present at about 2/3 of the posterior end of the tail (Figure 2F).

Xiphidiocercaria

Intermediate hosts: *B. siamensis* and *L. auriscularia*

Morphological characteristic: The body is elongated, oval-shaped, colorless and spinose. The oral sucker is circular and there is a stylet at the anterior of the body. The ventral sucker is present at the mid-body, globular and smaller than the oral sucker. The intestine is bifurcated and the pharynx is short. There are three pairs of penetration glands at about 2/3 of the body. The tail is slender and short. (Figure 2G).

The prevalence of cercarial infection was highest in Tha Ruea district (4.90%) due to numerous water resources, and conditions suitable for agriculture and fishery, followed by Bang Pahan (3.40%), Phra Nakhon Si Ayutthaya (3.35%), Phachi (3.07%), Uthai (2.00%), Phak Hai (1.49%), Bang Pa-in (1.35%), Maha Rat (1.00%), Ban Phraek (0.90%), Bang Sai (0.61%), Nakhon Luang (0.44%) and Wang Noi (0.00%), respectively (Figure 3).

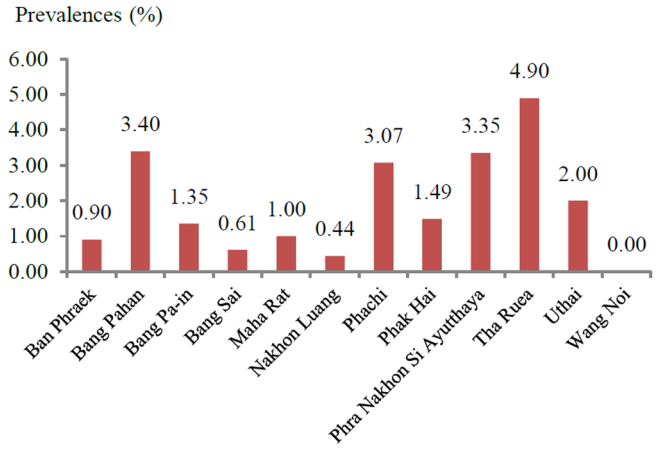

Figure 3 The prevalence of cercarial infections in each district.

Xiphidiocercaria were the dominant cercarial type infecting freshwater snails were (0.81%) followed by cercariae (0.37%), echinostome cercaria (0.27%), monostome cercaria (0.27%), parapleurolophocercous cercaria (0.14%), furcocercous cercaria (0.09%) and megalurous cercaria (0.05%).

In terms of cercarial diversity, xiphidiocercaria were discovered in two snail species, *B. siamensis* and *L. auricularia*. Previous reports indicated that this cercarial type was developed within the same snail species [16, 17] and encysted in fish, which is the secondary intermediate host, before maturing into the adult stage of Plagiorchiidae [18, 19]. Human infections by trematodes in Family Plagiorchiidae are very rare. Worldwide, only 11 cases have been reported in the literature [1, 2]. Moreover, a previous report found xiphidiocercaria was present in 3 snail families, Bithyniidae, Lymnaeidae and Thiaridae [20].

A previous report found parapleurolophocercous cercaria and xiphidiocercariae in freshwater snails with an overall prevalence of 13.8%. Further study indicated the prevalence of xiphidiocercaria infection was 6.6% in Nam Dinh province, Vietnam [20]. The freshwater snails found in Southeast Asia served as the intermediate host of various species, such as echinostome cercaria commonly found in viviparid and ampullarid snails, whereas the parapleurolophocercous cercariae generally infect Thiarid snails. These types of cercaria were reported as trematodes in Family Heterophyidae that infect various species of vertebrates, including human [6, 10]. Moreover, several report indicated the thiarid snails had shown a high susceptibility for parapleurolophocercous cercaria and xiphidiocercaria. As for monostome cercariae, they were found in bithynid snails in this study.

According to previous reports, this cercarial type usually infected similar snail species [10, 22]. Three snail species, *M. tuberculata, T. granifera* and *T. scabra*, serve as intermediate hosts for the megalurous cercaria, which have been reported as eye trematode of the avian in genus *Philophthalmus* [23-25].

The prevalence of cercarial infection in host can be used to estimate the relative roles of the intermediate and definitive hosts in the dissemination of eggs onto pasture. The limitation of this study was that the sampling sites did not cover all endemic provinces. Therefore, further studies concentrating on expanding the range of snail sampling sites is warranted.

CONCLUSION

This study revealed the high abundance of snails in Phra Nakhon Si Ayutthaya province, Thailand. Eight snail species, which were the intermediate hosts of trematodes, were *B. siamensis*, *Wattebledia sp.*, *C. helena*, *I. exustus*, *L. auricularia*, *T. scabra, F. martensi* and *F. polygramma*. The overall prevalence of cercarial infections was found to be 1.99%. It can be concluded that the diverse ecological conditions of the water resources of Phra Nakhon Si Ayutthaya support cercarial infection and distribution throughout this area.

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