

HABITATS AND TREMATODE INFECTION OF *BITHYNIA SIAMENSIS GONIOMPHALOS* IN UDON THANI PROVINCE, THAILAND

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Abstract. Determination of prevalence of trematode infection in *Bithynia siamensis goniomphalos*, the first intermediate host of *Opisthorchis viverrini*, was conducted from May 2014 to December 2015. Snails were collected from seven districts, namely, Kumphawapi, Kut Chap, Mueang Udon Thani, Nong Han, Nong Wua So, Phibun Rak and Prachaksinlapakhom, in Udon Thani Province, Thailand. *B. siamensis goniomphalos* snails were identified morphologically and examined for trematode infection by cercarial shedding. Seven hundred and sixty-four of 34,667 snails were infected with trematodes. Nine types of *O. viverrini* cercariae were identified, namely, amphistome, armatae, cystocercous, furcocercous, monostome, pleurolophocercous, virgulate, and unknown. The highest prevalent type of trematode infection was virgulate (11.53%), followed by armatae (1.35%). The prevalence of *O. viverrini* infection in Phibun Rak, Nong Wua So, and Kut Chap District was 0.22%, 0.08%, and 0.02%, respectively. Higher percent trematode infection was in small size snails (2-6 mm) than medium size snails (6.1-10 mm) (73.54% and 26.00%, respectively). Male snails were more infected (82%) than females (17%). Snails were most commonly found in water with pH 7.7 ± 1.0 , dissolved oxygen content 3.2 ± 1.7 mg/l, salt concentration 0.09 ± 0.12 ppt, conductivity 1.7 ± 0.1 mS/cm, and total dissolved solids 275.0 ± 0.2 mg/l. The highest prevalence of trematode cercariae was recorded in snails collected in reservoir dam (39.3%), followed by swamp (27.1%), canal (23.2%), rice paddy field (6.0%) and river (4.4%).

Keywords: *Bithynia siamensis goniomphalos*, *Opisthorchis viverrini*, prevalence, trematode

INTRODUCTION

One of the most critical problems in

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the Greater Mekong River sub-region is the infection of freshwater snails, which act as intermediate hosts for asexual multiplication of trematodes, especially *Opisthorchis viverrini*. The latter is an important causative agent for cholangiocarcinoma (CCA) (Haswell-Elkins *et al*, 1992; IARC, 1994; Vatanasapt *et al*, 2000). The prevalence of *O. viverrini* infection shows a strong positive correlation with the

occurrence of CCA, particularly in the rural areas of northeastern Thailand (Brockelman *et al*, 1986; Srivatanakul *et al*, 1991; Sriamporn *et al*, 2004; Rangsin *et al*, 2009; Kaewpitoon *et al*, 2015), where the highest incidence of CCA in the world occurs at Udon Thani Province (Khuhaprema *et al*, 2007).

The life cycle of *O. viverrini* requires two types of intermediate hosts, namely, snails, such as *Bithynia siamensis goniomphalos*, *B. funiculata* and *B. siamensis siamensis* (Brockelman *et al*, 1986; Sri-Aroon *et al*, 2005; Kulsantiwong *et al*, 2015) and fish, such as cyprinids (Sithithaworn and Haswell-Elkins, 2003; Upatham and Viyanant, 2003; Donthaisong *et al*, 2014; Tesana *et al*, 2014). Among the intermediate host snail species, *B. siamensis goniomphalos* is the most important, especially in hot spots of *O. viverrini* infection and CCA occurrence. In addition, a field study of *O. viverrini* infection in bithynid snails showed that *B. siamensis goniomphalos* has a higher prevalence of trematode infection than *B. siamensis* and *B. funiculata* (Kulsantiwong *et al*, 2015). The infection in these snails exhibited a higher prevalence in the dry season than the rainy season (Brockelman *et al*, 1986; Ngoen-klan *et al*, 2010; Kulsantiwong *et al*, 2013). The prevalence of *O. viverrini* infection in *B. siamensis goniomphalos* is low but very high in cyprinid fish (Sri-aroon *et al*, 2005; Pinlaor *et al*, 2013).

However, there is a lack of evidence of cercarial infection in *B. siamensis goniomphalos* in Udon Thani Province. The ecology of water bodies where the snails prefer to live includes water reservoir, canal and rice paddy field at depths of up to 50 cm (Brandt, 1974). In addition, freshwater quality optimal for bithynid snails is 3 to 420 NTU turbidity, 2.5-5 ppt salinity, pH 6.0-8.1, and 21.9-38.6°C water

temperature (Suwannatrai *et al*, 2011). The types of habitat of the snails might be one of the factors determining the prevalence of infection of this parasite in the snail hosts. Hence, the objective of this study was to evaluate the prevalence of trematode infection, especially of *O. viverrini* in *B. siamensis goniomphalos* in different types of water bodies in Udon Thani Province to serve as baseline data for future control measures of *O. viverrini* infection.

MATERIALS AND METHODS

Samples

Bithynia siamensis goniomphalos samples were collected from May 2014 to December 2015 manually for a period of 5 minutes/site per person from rocks, soil, mud and sandy ground and using scoop sieves at locations presenting with grass and leaves on clean or muddy surfaces or with decaying leaves. The snail collections were conducted at 55 localities in 20 rice paddy fields, 20 reservoirs or dams, 5 swamps, 7 canals and 3 rivers in 7 districts in Udon Thani Province, namely, Kumphawapi, Kut Chap, Mueang Udon Thani, Nong Han, Nong Wua So, Phibun Rak and Prachaksinlapakhom (Fig 1). The snails were cleaned, air-dried, separately labeled according to site and locality, stored in plastic bags and transported to the laboratory.

The physicochemical properties of water at collection sites were analyzed using an Extech DO700 Portable Dissolved Oxygen instrument (FLIR Commercial Systems, Nashua, NH). The collected snails were identified based on shell morphology for species following available keys and descriptions (Brandt, 1974; Chitramvong and Upatham, 1989; Chitramvong, 1991, 1992; Kulsantiwong *et al*, 2013; Tesana *et al*, 2014). The infected

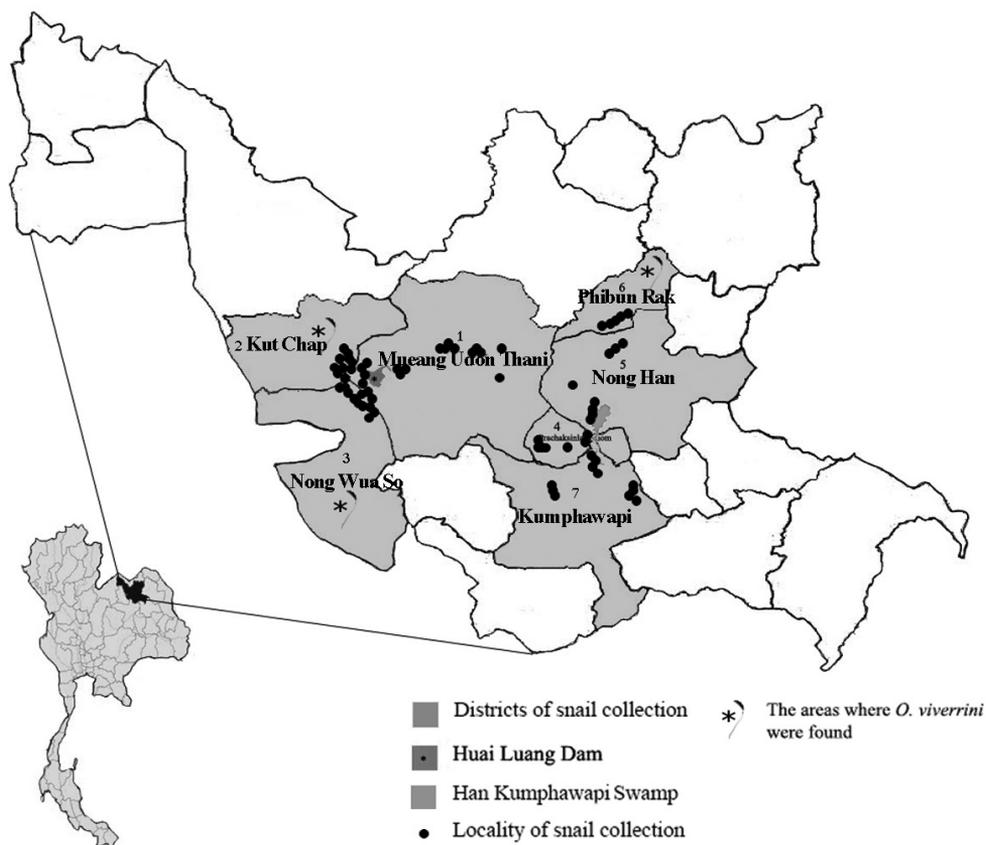


Fig 1–Map of Udon Thani Province, Thailand showing snail collection districts and sites.

snails were divided into two groups based on gender and were randomly sampled for measurement of shell size using an electronic digital caliper (Tianlun Hardware Tool Manufacture, Zhangjiagang, Jiangsu, China): small (2.0-6.0 mm) and medium (6.1-10.0 mm) (Kulsantiwong *et al*, 2013; Prasopdee *et al*, 2015).

Trematode collection and identification

Dechlorinated tap water was used to clean the snails and then infected trematodes were collected using a cercarial shedding method (daytime and nighttime shedding). Snails were placed separately in small plastic cups containing 10 ml of dechlorinated tap water. Shedding was

induced under 25 W electric light bulb illumination for 3 to 4 hours at room temperature (25°C) during the daytime. At night time, black covers were used to achieve total darkness. Each snail was carefully observed for cercariae under a stereomicroscope for daytime and nighttime shedding. The live cercariae were stained with 0.1% Fast Red B (Sigma, St Louis, MO), then observed under a compound microscope and photographed. The types of cercariae were identified based on the available morphological description (Schell, 1970; Frandsen and Christensen, 1984; Adam *et al*, 1993). *O. viverrini* was confirmed using PCR-based assay employing species-specific

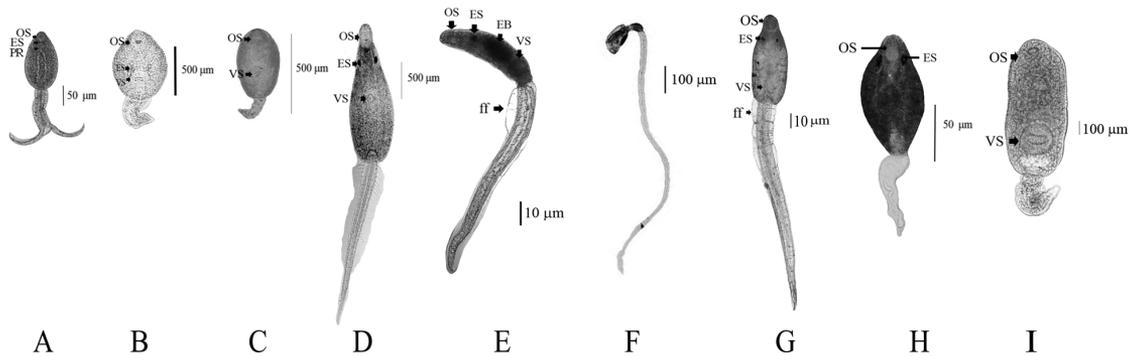


Fig 2– Type of cercariae shed from *Bithynia siamensis goniomphalos*. (A) Furcocercous, (B) amphistome, (C) virgulate, (D) armatae, (E) *O. viverrini*, (F) cystophorous, (G) parapleurolophocercous, (H) monostome, and (I) unknown. EB, excretory bladder; ES, eye spot; ff, fin fold; OS, oral sucker; VS, ventral sucker.

primers OV-6F (5'-CTGAATCTCTCGTTT-GTTC-3') and OV-6R (5'-GTTCCAGGT-GAGTCTCTCTA-3') (Wongratanacheewin *et al*, 2001).

Statistical analysis

Pearson correlation coefficient was used to gauge the correlation between density of snail population and prevalence of trematode infection (a p -value <0.05 is considered statistically significant). Descriptive results were expressed as mean \pm standard deviation (SD) of size, density of snail population from the difference habitats and quality of water. Statistical analysis was performed using SPSS version 16.0 software package (SPSS, Chicago, IL).

RESULTS

Seven hundred and sixty-four (15.08%) of 34,667 *B. siamensis goniomphalos* were infected with trematodes, mainly (3.6%) those in Nong Han District, followed by Nong Wua So District (2.98%), Kumphawapi District (2.09%), Mueang District (2.0%), Prachaksinlapakhom District (1.96%), Phibun Rak District (1.8%), and Kut Chap District (0.86%). Only three districts, namely, Phibun Rak,

Nong Wua So and Kut Chap, had *O. viverrini* infection of 0.22%, 0.08% and 0.02%, respectively, all located near Huai Luang Reservoir.

The highest prevalence (39.3%) of trematode were recorded in snails collected in reservoir/dam, followed by swamp (27.1%), canal (23.2%), rice paddy field (6.0%), and river (4.4%). The mean snail density (number/manpower/5 minutes) of *B. siamensis goniomphalos* in different localities in the 7 districts surveyed of Udon Thani Province was as follows: in the river of Kumphawapi District was 105 ± 22 , reservoirs/dams of Kut Chap and Nong Wua So District 275 ± 46 and 441 ± 37 respectively, canals of Mueang and Prachaksinlapakhom District 355 ± 51 and 75 ± 2 , respectively, swamps of Mueang, Prachaksinlapakhom and Phibun Rak District 355 ± 50 , 83 ± 22 and 215 ± 69 , and rice paddy fields of Prachaksinlapakhom, Nong Han and Phibun Rak 78 ± 23 , 103 ± 52 and 167 ± 52 , respectively. Of the 378 infected snails sampled, 278 (74%) were in small size and the remaining in medium size group, with 312 (82%) being females. The relationship between snail density and prevalence of trematode infection is not significant (Fig 2).

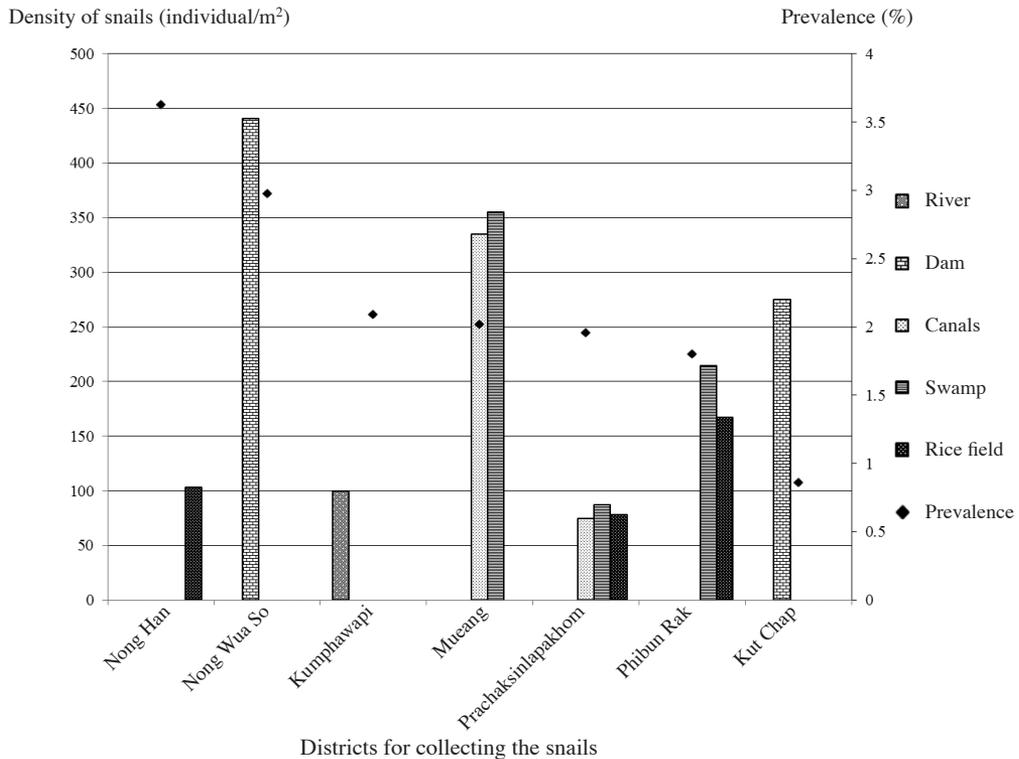


Fig 3—Density of snails and prevalence of trematode infection in *Bithynia siamensis goniomphalos* in various habitats of Udon Thani Province, Thailand.

Nine types of cercariae infecting *B. siamensis goniomphalos* were identified: amphistome (0.05-0.28%), armatae (0.06-0.95%), cystophorous (0.03-0.06%), furcocercous (0.16-0.48%), monostome (0.11-0.89%), *O. viverrini* (0.02-0.22%), parapleurolophocercous (0.09%), virgulate (0.08-2.92%), and unknown (0.05%) (Fig 3). The morphological characteristics of all cercariae are presence of oral and ventral suckers, except for cystophorous that have tail bulbs, cyst-like and appendices, parapleurolophocercous and *O. viverrini* clear fin folds, and furcocercous is a forked tails. *O. viverrini* was confirmed by a PCR-based assay (Fig 4).

Water temperature ranged from 20.2 to 40.2°C with pH of 6.5 to 10.5. Dissolved oxygen content was 1.5-25.7 mg/l, turbidity 129-763 mg/l, conductivity 609-

1,870 µS/cm, and salinity 0.01 to 3.49 ppt, parameters that did not correlate with *B. siamensis goniomphalos* density.

DISCUSSION

B. siamensis goniomphalos is the first intermediate host of liver fluke in Thailand, especially in the northeast area (Brandt, 1974). This study is the first report of the prevalence of trematode infection in *B. siamensis goniomphalos* in the northeast province of Udon Thani. It was noticeable that the three districts, namely, Phibun Rak, Nong Wua So and Kut Chap, where *O. viverrini* was detected, are located near Huai Luang Reservoir. the major source of food for people in these diastriacts. The natural life cycle of *O. viverrini* is complete in these districts as the localities

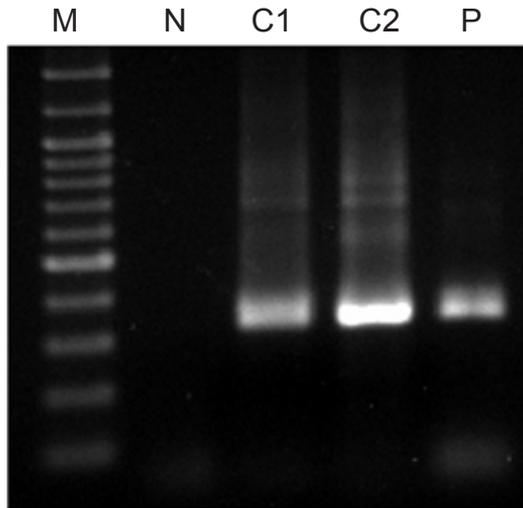


Fig 4—*Opisthorchis viverrini* cercariae-specific amplicon (330 bp). PCR was conducted as described by Wongratanacheewin *et al* (2001). Lane M, DNA size markers; lane N, negative control (sterile distilled water); lanes C1 and C2, *O. viverrini* cercariae; P, positive control (adult *O. viverrini*).

surveyed contain the first and second intermediate and definitive hosts. Recent studies reported 1.07-6.93% prevalence of trematode infection in various freshwater snails in northeast Thailand (Kiatsopit *et al*, 2012; Chontanarith and Wongsawad, 2013; Kulsantiwong *et al*, 2015; Mohammed *et al*, 2016). In this study, several mollusk species were observed in the same habitat but the infected snails were only *B. siamensis goniomphalos*, with percent of virgulate infection similar to a previous report (10.90%) in nine provinces of northeast Thailand, but higher than in Lao PDR (6.58%) (Kiatsopit *et al*, 2014). However, our value was slightly lower than the reports of 3.11%, 6.80% and 1.64% in the rainy, cool, and hot season, respectively in Phang Khon District, Sakon Nakhon Province, Thailand (Namsanor *et al*, 2015). Thus, these surveys indicate that virgulate infection of *B. siamensis goniomphalos*

is the most common type of trematode infection, but differences in prevalence are the consequences of various factors, such as time of collection, season, type of trematode, and water quality. In addition, the size of snails affected the prevalence of trematode infection as large size *B. siamensis goniomphalos* has significantly higher prevalence of *O. viverrini* than medium size individuals (Kiatsopit *et al*, 2012). The nine morphologically distinguishable types of trematode were observed in *B. siamensis goniomphalos* agreed with results of a previous report in Khon Kaen Province (Tesana *et al*, 2014).

Suwannatrai *et al* (2011) reported that the presence of live *B. siamensis goniomphalos* is dependent on various physical characteristics of the water habitat, *viz.* temperature, dissolved oxygen concentration, pH, and turbidity, with water salinity of 2.5-5.0 ppt being the most important parameter. On the other hand, we failed to discern any effect of these factors on *B. siamensis goniomphalos* density. Clearly, this needs further investigation.

In Udon Thani Province, the prevalence of the parasite infection in snails and fish has been reported (Waikagul, 1998; Kulsantiwong *et al*, 2013; Kiatsopit *et al*, 2014). Even though there was no previous report of *O. viverrini* metacercariae infection in local fish population of Udon Thani Province, we were able to observe *O. viverrini* in snails host from Huai Luang Reservoir Dam, Udon Thani Province in 2014 (unpublished data).

In summary, the study determined the prevalence of *B. siamensis goniomphalos* in seven districts (Kumphawapi, Kut Chap, Mueang Udon Thani, Nong Han, Nong Wua So, Phibun Rak and Prachaksinlapakhom) of Udon Thani Province. The distribution of these snails varied depending on the ecology of the water

bodies and were infected with nine types of trematode cercariae.

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