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 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
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.

**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 ± 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%), Phbun 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 (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%), virgulare (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-likes 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 districts. The natural life cycle of *O. viverrini* is complete in these districts as the localities
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; Chontananarth 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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