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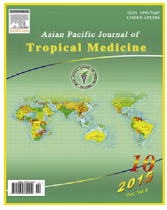

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Bithynia siamensis goniomphalos, the first intermediate host of *Opisthorchis viverrini* in Thailand

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ABSTRACT

Opisthorchiasis caused by *Opisthorchis viverrini* (*O. viverrini*) remains as medically important problem in Thailand especially in the north-eastern part. Infection with this parasite can lead to cholangiocarcinoma improvement. The highest prevalence of *O. viverrini* infection has been found in the Northeast Thailand and is associated with the high incidence rate of cholangiocarcinoma. To complete the life cycle of *O. viverrini*, the freshwater snails namely *Bithynia funiculata*, *Bithynia siamensis siamensis* and *Bithynia siamensis goniomphalos* (*B. s. goniomphalos*) are required to serve as the first intermediate host. Within these snails group, *B. s. goniomphalos* is distributed concisely in northeast Thailand and acts as the majority snail that transmitted the opisthorchiasis in this region. This study described the information of *B. s. goniomphalos* which research are needed for understanding the biology, distribution, transmission and factors influencing on the infection of the snail vector of this carcinogenic parasite.

1. Introduction

Opisthorchis viverrini (*O. viverrini*) is the human liver fluke caused opisthorchiasis, the food-borne disease that establishes a serious public health problem in Southeast Asia where consists of Thailand, Lao People's Democratic Republic, Cambodia and Vietnam [1]. *O. viverrini* infection is essential to be familiar with because the chronic infection can induce inflammation and pathological changes in biliary tract, after that numerous numbers of infected people becomes seriously morbidities such as hepatobiliary diseases and cholangiocarcinoma [1–3]. Moreover, *O. viverrini* is only the liver fluke that has been identified as class I carcinogen [4]. Although infections are identified throughout Southeast Asia, the most widespread is northeast Thailand, where high prevalence coexists with a high incidence of cholangiocarcinoma [5].

For life cycle, this parasite necessitates three-hosts to complete. Freshwater snails of three species, *Bithynia siamensis goniomphalos* (*B. s. goniomphalos*), *Bithynia siamensis siamensis* (*B. s. siamensis*) and *Bithynia funiculata* (*B. funiculata*), play role for its first intermediate host in northeastern, central and northern of Thailand, respectively. Cyprinoid fish approximately 18 species act as its secondary intermediate host. The definitive hosts are cats, dogs, and various fish-eating mammals including humans, which acquire infection through the consumption of improperly cooked fish [6–10].

After *Bithynia* snail ingested *O. viverrini* egg, the juvenile worm multiplied by asexual reproduction in bithyniid snails to produce numerous cercaria then furthered to infect cyprinoid fish, the second intermediate host to be metaceraria. After that, human who plays role as definitive host became infection by consuming raw or undercooked fish [11]. The prevalence of infection was high in humans and fish intermediate host although the infection rate in snail intermediate host was very low [12–14]. For that reason, we interested to inform in majority snail which plays role in parasite growth, development and transmit parasitic infection in northeast Thailand.

B. s. goniomphalos is distributed concisely in northeast Thailand where the highest incidence of cholangiocarcinoma was occurrence. Consequently, this snail is served as the majority snail that transmitted the opisthorchiasis in this region. In the previous reports, both field survey and laboratory indicated that the susceptibility for *O. viverrini* infection of *B. s. goniomphalos* is lowest when compared to the other intermediate snail hosts, *Bithynia funniculata* and *B. s. siamensis* [15]. Although, the rate of *O. viverrini* infection in intermediate snail host is very low, one infected snail can produce massive cercaria for next step infection to the cyprinoid fish [16].

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The information of this review will enable us to better understand the natural of *B. s. goniomphalos* including the life cycle, distribution and contributions to disease transmission. Understanding the intermediate snail host is necessary for developing and achieving programs to control opisthorchiasis and cholangiocarcinoma. The information of snails intermediate host are research needed for understanding the transmission, distribution and control of this carcinogenic parasite.

2. Biology of *B. s. goniomphalos*

2.1. Classification

Bithynia snail was classified in phylum Mollusca, class Gastropoda, subclass Prosobranchia, order Mesogastropoda, superfamily Rissooidea, family Bithyniidae. In Thailand, the snails in family Bithyniidae are identified based on shell morphology and divided into 3 genera; *Bithynia*, *Hydrobioides* and *Wattebledia*. The genus *Bithynia* consists of two subgenera, *Digoniostoma* and *Gabbia*. However, some references recognized *Gabbia* as a genus [17]. Three species belonging to the genus *Bithynia* act as the first intermediate hosts of *O. viverrini* are namely *B. funniculata*, *B. s. siamensis* and *B. s. goniomphalos* [18,19]. Twelve species of bithyniid snails in the family Bithyniidae was reported in Thailand [16–18] (Table 1).

2.2. Morphology

B. s. goniomphalos is a dextral (shell coiled to the right) and organized its body (respiratory, excretory and reproductive) opening on the right side. This snail is a relatively small species of freshwater snail with gills and an operculum. The shell morphology is subovately conic and reddish brown color with a wide umbilicus. The spires of the adult snail consist of 4 whorls with an eroded apex. The height of shell is usually no larger than 10.2–14.9 mm long and the width of the shell is 5.6–8.5 mm. The operculum is concentric with spiral nucleus [18,19].

2.3. Livelihood

B. s. goniomphalos is dioecious (separately sexes). The male copulatory organ or verge which can distinguish sexes is located on the top header, when the head foot of the snail lean out from the shell during the snail attempt to right itself, the verge can be seen clearly. On the head foot, 2 long tentacles are pointed out. This snail can produced two generations per year [14].

B. s. goniomphalos may feed both by filter feeding using its gills to filter suspended algae from the water and by grazing on the substrate. This snail uses its teeth or radula to graze or erode on algae or organic substance as well as the eggs of flukes.

2.4. Habitat of *B. s. goniomphalos*

This snail lives in slow-running freshwater habitat such as muddy rivers and irrigation canals and standing-water bodies such as artificial and natural ponds, rice fields, lakes and swamps [20,21]. However, habitat types where this snail was more dominant are rice paddies [22]. It is commonly found on and in the mud, mixed sand-mud substrate [19], on rocks as well as on vegetation [14]. *B. s. goniomphalos* snail was observed in shallow water but is also found at all depths up to 10 m [23]. It lives habitually in areas with a paucity of aquatic plants (*Typha angustifolia*, *Ipomoea aquatic*, *Neptunia oleracea* and *Nymphaea lotus*) and only a few macrophytes by crawling on these aquatic plants or on the surface of the soil, but no snails were found in areas with a high density of water plants or in rapidly running water [20]. Moreover, this species prefers a substrate of algae or other water plants such as lotus leaves [19].

Field survey in Khon Kaen province reported that this snail habitats water with temperature (°C) of 23.08–34.22, dissolved oxygen (mg/L) range between 1.97 and 8.67, conductivity (mS/cm) between 0.27 and 8.47, total dissolved solids (g/L) of 0.14–4.99, salinity (ppt) range between 0.12 and 4.22 and pH range 6.23–8.65 [21]. Field survey in Khorat basin consists of 12 provinces in northeast Thailand reported this snail habitats water with temperature (°C) of 21.9–38.6, dissolved oxygen (ppm) range between 0.01 and 6.47, conductivity (mS/cm) varied between 0.12 and 40.2, salinity (ppt) range between 0.05 and 22.10 and pH range 6.02–8.07 [20]. Similar profiles of physico-chemical characteristics of fresh water habitat were recorded except dramatically difference in salinity and conductivity levels which direct variation by salinity. This suggest that northeastern part of Thailand is covered with surface salt over one third of the region [24] which in turn results in high salinity concentration in reservoirs. For inland freshwater, the increasing salinity was considered as a serious environment for survival of aquatic organisms both animals and plants [25,26]. However, it has been reported that moderate saline water is helpful to some aquatic animals such as snails and clams [27–29]. However, habitat types, abiotic and biotic factors are impacted for the physico-chemical characteristics of fresh water habitat for *B. s. goniomphalos* and potentially influenced the

Table 1

Twelve species of *Bithyniid* snails in the family Bithyniidae found in Thailand.

Genera and subgenera	Specific epithet	Distribution region in Thailand
Bithynia (Digoniostoma)	*Funniculata*	North
B. (Digoniostoma)	*Siamensis siamensis*	Western and central
Bithynia (Digoniostoma)	*Siamensis goniomphalos*	Northeast Thailand, Lao PDR, Southern Vietnam
Bithynia (Digoniostoma)	*Pulchella*	North
Bithynia (Gabbia)	*Pygmaea*	North
Bithynia Gabbia)	*Wykoffi*	North
Bithynia (Gabbia)	*Walkeri*	Supanburi province
Bithynia (Gabbia)	*Erawanensis*	Central
Hydrobioides	*Nassa*	Central
Wattebledia	*Siamensis*	Central
Wattebledia	*Crosseana*	Northeast
Wattebledia	*Bashi*	Southern

distribution and abundance of snail populations [22]. To date, no data reports about the most appropriate physico-chemical characteristics of fresh water habitat for *B. s. goniomphalos*.

3. Medical importance as the first intermediate host

B. s. goniomphalos play role as the amplifying host of the human liver fluke, *O. viverrini*. When the adult worms produce embryonated eggs and the eggs pass through the bile ducts through the lumen of the intestines and are excreted from the definitive host via the feces and polluted into the water, the *B. s. goniomphalos* was infected by ingest the eggs. The miracidia in the eggs are hatched and growth in the digestive tract of the snail. The alterations of digestive gland induced by *O. viverrini* infection were investigated [30]. In snail host, the miracidia transform into sporocysts, rediae and germinal cells in rediae produce cercariae. The mature cercaria are released out from snails and become free swimming stage when they are activated by the light. The optimal light conditions for *O. viverrini* cercarial shedding was determine in the laboratory which indicated that a light intensity of 1 000 lx (approximately 40 W, 10 in) for 2 h is optimal for *O. viverrini* cercaria emergence from naturally inflected *B. s. goniomphalos* [31]. The time of cercarial emergence was not consistent in different seasons, occurring between 08.00 and 10.00 h during the hot-dry season and between 12.00 and 14.00 h during the rainy and cool-dry seasons. However, the cercarial output was highest in the hot-dry season [32]. The maximum cercaria shed from one snail were 1 728 cercaria in a day. The total output from one snail was 27 692 [16]. The development of the *O. viverrini* intramolluscan stages takes 8–10 wk [11]. Free swimming cercaria penetrate and discard their tail before they encyst to be metacecaria in the body of cyprinoid fish such as *Cyclocheilichthys* spp., *Puntius* spp., *Hampala dispa*, *etc*. [12,33]. Human are infected when eat raw or improper cooked fish which contain metacercariae. The entire life-cycle of *O. viverrini* requires from 4 to 4.5 months and the worms have a life span of 25–30 years [11].

Moreover, *B. s. goniomphalos* in Thailand has been reported as the intermediate host to many different species of trematode by cercarial shedding technique. Types of trematode cercaria found were *O. viverrini*, Virgulate, Ubiquita, Furcocystocerous, Strigea, Armatae, Gymnocephalous, Monostome, Amphistome including unidentified cercariae [8,34].

4. Factors influence *O. viverrini* cercaria infection

The successfully infection between parasites and their intermediate snail hosts is influenced by multi-factors. Sewage pollution is one of the important factors to concern because *O. viverrini* eggs contaminated via feces to the environment. So determination of coliform bacteria, common bacteria found in feces of human and other warm blood animals, can address the presence of fecal contamination [35,36]. Natural water reservoirs in Khon Kaen province was observed about correlation of fecal bacterial contamination and *O. viverrini* infected *Bithynia* snails. The results indicate that fecal bacterial contamination in natural water reservoirs is an important indicator of seasonal transmission of *O. viverrini* eggs to snail intermediate hosts [37].

Habitats is associated with the ability of *O. viverrini* infection in *Bithynia* snail as the rice field aquaculture has the highest prevalence of *B. s. goniomphalos* infected with cercaria of *O. viverrini* [38]. The authors [32] studied about seasonal factor by collected of *B. s. goniomphalos* on three occasions from Vientiane Province, Lao PDR: in hot-dry season, rainy season and cool-dry season. The results showed prevalence of *O. viverrini* in field collected *B. s. goniomphalos* was highest in the cool-dry season. In addition, small snails are more susceptible for parasitic infection.

A recent study [39] investigated about factors that influence *O. viverrini* infection by infected *B. s. goniomphalos* in laboratory. The results showed temperature was reported as the strongly dependent factors for *O. viverrini* infection in *B. s. goniomphalos*. A temperature of 34 °C was optimal for obtaining the highest infection rate. However, the infection rates between female and male snails displayed similar. And small sized snails were more susceptible to infection than larger snails. On the other hand, the previous research [14] reported that *O. viverrini* infection was found in *B. s. goniomphalos* which shell larger than 8 mm in length but smaller snail were not found.

The immune system of the snail host is the never disregard factor. Functional studies of genes and gene products potentially involved in immune mechanisms implicated in the ability of the parasite to successfully infect its snail intermediate host has been study. The transcriptomes of uninfected and *O. viverrini*-infected *B. s. goniomphalos* was characterized. *Bithynia* specimens infected by *O. viverrini* displayed the highest down-regulation of proteases but significantly up-regulated transcription of genes encoding heat-shock proteins [40]. According to the protein expression analysis, *O. viverrini* infection down regulates the expression of oxidoreductases and catalytic enzymes, while stress-related and motor proteins are upregulated [41].

5. Conclusion

B. s. goniomphalos analyzed here distributed concisely in northeast Thailand where the highest incidence of cholangiocarcinoma was occurrence. Surprisingly, both field survey and laboratory indicated that the susceptibility for *O. viverrini* infection of this snail species is lowest when compared to the other intermediate snail hosts, *B. funniculata* and *B. s. siamensis*. This phenomenon is much complicate and also need to be explored. Although, the rate of *O. viverrini* infection in snail is very low, one infected snail can produce enormous cercaria to infect many cyprinoid fish in the next step.

For *O. viverrini* infection, both external (abiotic and biotic composition in environment) and internal factors are influence for the success of parasitic infection. Genetic variations of the *Bithynia* snails may differ in their resistance and susceptibility to parasitic infections. The better perceptive for prevention and control opisthorchiasis require a good understanding for the external and internal factors constituents influenced parasitic infection also host/parasite interaction. The genetic differentiation of the snail host is the new target for investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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