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Opisthorchis viverrini infection in the snail and fish intermediate hosts in Central Vietnam

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ABSTRACT

Opisthorchis viverrini, a carcinogenic fish borne fluke, requires freshwater snails and fish as intermediate hosts. Opisthorchiasis is endemic in parts of Southeast Asia, including Central and South Vietnam. In this region the transmission by intermediate hosts has received little attention. Therefore, freshwater snails and wild fish from Bau My Tho, an opisthorchiasis endemic area in Binh Dinh Province were collected for examination of *O. viverrini* cercariae and metacercariae, respectively. A total of 12,000 snails belonging to six families, of which 1616 *Bithynia* snails representing *Bithynia siamensis goniomphalos* and *Bithynia funiculata*; as well as 754 fish representing 12 species were examined. Shedding of *O. viverrini* cercariae was observed only in *B. s. goniomphalos* and *B. funiculata*, at infection rates of 0.86% and 0.14%, respectively. *O. viverrini* infection in *Bithynia* spp. was significantly associated with the habitat but not with the species and the shell size of *Bithynia* spp. *O. viverrini* metacercariae were found in 10 fish species representing both Cyprinidae and non-Cyprinidae families. The prevalence of *O. viverrini* infection in fish was significantly associated with species. *Carassius auratus*, a fish species commonly eaten raw, *Rasbora aurotaenia* and *Puntius brevis* had the highest prevalence of 74.0%, 55.8% and 31.6%, respectively. Sharing of the same snail and fish intermediate host species was found for *O. viverrini* and a *O. viverrini* duck-genotype that are sympatric in the study region. This study is the first to report on the intermediate host species of *O. viverrini* in Central Vietnam and indicates a high risk of acquiring opistorchiasis when eating raw fish dishes.

1. Introduction

The Southeast Asian liver fluke *Opisthorchis viverrini*, a fish-borne trematode, is the causative agent of opisthorchiasis. This condition is a major public health problem in the Greater Mekong Region, including northeast Thailand, and parts of Cambodia, Lao PDR and Vietnam (WHO, 2008, 2011). Although most cases of opisthorchiasis are asymptomatic, the condition can lead to cholangitis, cholecystitis and even cholangiocarcinoma, a malignant tumour of the bile duct (Sripa 2003; Sripa et al., 2012). It is estimated that more than 10 million people are infected with *O. viverrini*. In Thailand, where the disease has been studied extensively, the economic loss caused by opisthorchiasis is estimated at 120 million USD annually (Andrews et al., 2008; Sithithaworn et al., 2012; Sripa et al., 2007).

The life cycle of *O. viverrini* involves bithynid freshwater snails (Ditrich et al., 1990) as the first intermediate hosts and more than 80 species of Cyprinid fish and at least 13 fish species belonging to other families of freshwater fish as the second intermediate hosts (Sithithaworn and Haswell-Elkins, 2003). Humans acquire the infection by consumption of raw or undercooked fish that contains metacercariae (Kaewkes 2003). Dogs and cats and other fish-eating mammals can act as reservoir hosts (Aunpromma et al., 2012; Kaewkes 2003). The disease is strongly related to the culinary tradition of raw fish consumption. In Vietnam, *O. viverrini* is present in the central and southern parts of the country, while the related

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Clonorchis sinensis occurs in the North (Nguyen and Le 2011). The first cases of opisthorchiasis were identified in Central Vietnam in the 90 s (Chuong and Tuan, IMPE website accessed 2016). However, the epidemiology of this parasitic disease has received little attention in this region. In a recent study, we estimated the prevalence of opisthorchiasis at 11.4% in a commune of Binh Dinh Province; almost half of the local population, reporting eating raw fish dishes was at risk for the infection (Dao et al., 2016). Few data are available on the intermediate host species responsible for the transmission of *O. viverrini* in Central Vietnam. The epidemiological situation in Central Vietnam is further complicated by the existence of a *O. viverrini* – like species that was found in the liver of ducks in this region (Dao et al., 2014; Dorny et al., 2015). Given the close genetic similarity of this parasite to human isolates of *O. viverrini*, sharing of the same intermediate host species is plausible. A better knowledge of the epidemiology, including transmission by intermediate hosts is needed for identifying risk factors and developing control measures for opisthorchiasis.

The aim of the current study was to identify the snail and fish intermediate host species and investigate the prevalence and intensity of *O. viverrini* infection in these intermediate hosts in an opisthorchiasis endemic area in Central Vietnam. In addition, the association between prevalence and intensity of *O. viverrini* infection, and host size as well as snail habitat was determined.

2. Materials and methods

2.1. Study area

Bau My Tho (My Tho Lake) (14°13′23″N 109°9′16″E), located in Binh Dinh Province (Fig. 1), an endemic area of opisthorchiasis in Central Vietnam (Dao et al., 2016), was selected for investigating *O. viverrini* infection in snails and fish.

"Bau" or lake is a small freshwater reservoir commonly present in lowland communes in the central coastal area of Vietnam and consists of a complex of irrigation canals, rice fields and small ponds. Flooding occurs all over the "bau" in the rainy season that spans from September to December, turning the area into a big lake during this period. Freshwater activities of the commune, such as rice and vegetable production, and duck farming, typically occur in the "bau". A wide diversity of freshwater snails and wild fish species inhabit the "bau". One of these fish species, the "diec" (*Carassius auratus*) is preferred for preparation of the traditional raw fish dish in Binh Dinh province (Dao et al., 2016).

In 2014 (March to May) and 2015 (January to May), snails and wild fish were collected twice a month in Bau My Tho for identification and examination of *O. viverrini* (-like) cercariae and metacercariae.

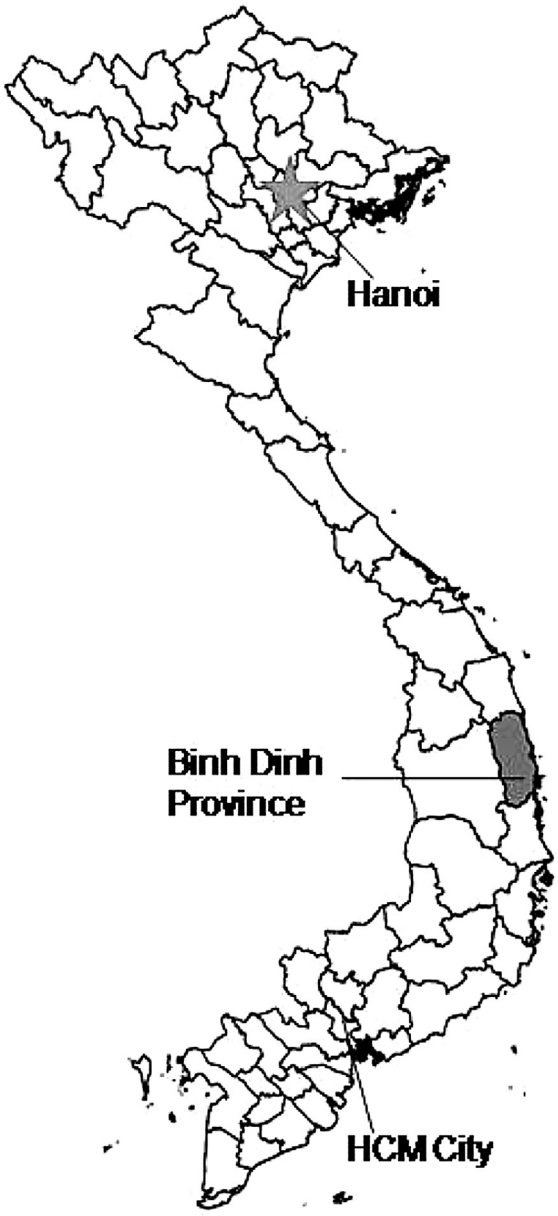

Fig. 1. Map of Vietnam showing Binh Dinh Province where Bau My Tho (My Tho Lake) is located.

2.2. Collection and examination of snails

Snails were collected by hand picking on the banks of irrigation canals, rice fields and ponds in the Bau My Tho following the snail sampling method of Bui et al. (2010). A distance of approximately 3 km around the lake was covered for snail sampling; every 10 m, an area of about one square meter was inspected and all visible snails were collected. Snails from each collection point were kept separately for examination. Snails were transported in a cool box to the laboratory of the Sub-Animal Health Department of Binh Dinh province where they were identified using keys for freshwater snails (Chitramvong 1992; Upatham et al., 1983). *Bithynia* snails were identified at sub-species level. *Bithynia siamensis goniomphalos* and *Bithynia funiculata* have a sub-oval, conic shell. The umbilicus of *B. s. goniomphalos* is relatively wide and deep with a weak carina, whereas the umbilicus of *B. funiculata* is funnel-shaped with a strong carina. In addition, shell sizes (length and width) of infected snail species were measured. All collected snails, both *Bithynia* and other species were transferred to transparent plastic cups containing tap water. First, 10 snails of the same species were pooled for observing cercarial shedding. If pleurolophocercous cercariae were seen, individual snails were transferred to cups for shedding. A snail was considered infected with *O. viverrini* (-like) when pleurolophocercous cercariae (Adam et al., 1995; Kiatsopit et al., 2016) were observed. Pleurolophocercous cercariae were differentiated from parapleurolophocercous cercariae from *Haplorchis* spp. and other cercariae based on morphological differences (Nissen 2012; Schell 1985). For molecular confirmation, pleurolophocercous cercariae from each infected snail were randomly selected and preserved in RNA*later*™ buffer (Qiagen, Texas USA, cat No./ID: 76104) at 4 °C in pools of one, five, or 100 (Webster, 2009). The total cercarial shedding over 24 h from each positive snail was collected and counted.