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Fish-borne Parasitic Zoonosis in Lower Mekong Basin Countries: Review

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ABSTRACT

Fish borne parasitic infections have been important public health problems in many parts of the world, particularly in countries lower Mekong regions for example; Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia and Myanmar. Currently, many reports indicate that metacercariae of pathogenic trematodes are showed in freshwater fish commonly small liver fluke, *Opisthorchis viverrini*; minute intestinal flukes (MIF); family of Heterophyidae. In Thailand, the epicenter of this disease is located in the northeast region, where high a prevalence of opisthorchiasis coexists with a high incidence of cholangiocarcinoma (CHCA), a major primary carcinoma of the liver with a very poor prognosis. Fish-borne trematode metacercariae are found in a variety of fish species, relating to carcinogenic liver fluke and minute intestinal flukes. This review aimed to summarize the association of fish species (second intermediate Host) and metacercariae species in lower Mekong basin countries. Also, this review might provide evidence leading to improved public health awareness for surveillance and control of FBT contamination and contribute to filling the gap of information necessary for the control and prevention of fish-borne trematode zoonotic infections in the lower Mekong region countries.

INTRODUCTION

Zoonotic trematode (ZT) infections are an important public health problem in many Asian countries, including Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia, Thailand, the Philippines, China, Taiwan, and the Republic of Korea (Korea) [1-4]. Especially, fish borne trematodes (FBT) provoke remarkable morbidity in residents of these countries and cause serious economic damage in the industry of fish aquaculture [5,6]. Currently, more than 45 million people estimated to be infected [7]. Small liver flukes, Opisthorchiidae and minute intestinal flukes (MIF), Heterophyidae are highly prevalent in Southeast Asian countries. Embryonated eggs containing miracidium are discharged in the biliary ducts and passed in the stool. Eggs are ingested by a suitable snail intermediate host (First intermediate host). Within the snail, eggs hatch to release miracidia, which transform to sporocysts [8]. Then sporocysts undergo asexual reproduction to give rise to rediae and finally cercariae. Cercariae are released from the snail and after a short period of free-swimming time in the water, it penetrates the skin of freshwater fish (second intermediate host), where it encysts as a metacercaria [9]. Humans are infected through ingestion of undercooked or raw freshwater fishes. The metacercariae excyst in the duodenum and ascend the biliary tract through the ampulla of Vater. Maturation to adult worms of trematode infection inhabits the intra and extrahepatic biliary system [10]. The human liver flukes, *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis* remain important public health problems in many parts of the world, particularly in Asia. [11,12]. *Clonorchis sinensis* is endemic in southern China, Korea and northern Vietnam, whereas *O. viverrini* is endemic in the Lower Mekong Basin, including Thailand, Lao People's Democratic Republic (Lao PDR), Cambodia and central Vietnam and Myanmar [12,13]. *Opisthorchis felineus* is found in Central–Eastern Europe.

It has been known that more than 50 million people are infected with intestinal trematodes, and about 70 trematode species are involved in human infections around the world [14,15,16]. Particularly, they are prevalent in Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia, Thailand, Myanmar, the Philippines, China, Taiwan, and the Republic of Korea (Korea). [17,18,19,20]. Countries of lower Mekong regions are highly alarmed the spread of fish borne trematode infections i.e. small liver flukes and minute intestinal flukes especially in Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam, Cambodia and Myanmar. [21,22]. Fish-borne zoonotic trematodes (FZT) especially small liver flukes (Opisthorchiidae) and minute intestinal flukes (Heterophyidae) are highly prevalent in that regions [23]. These two flukes have similar life cycles and involved two intermediate hosts to complete their life cycle [24]. The first intermediate hosts are snails and the second intermediate hosts are small freshwater fishes [25].

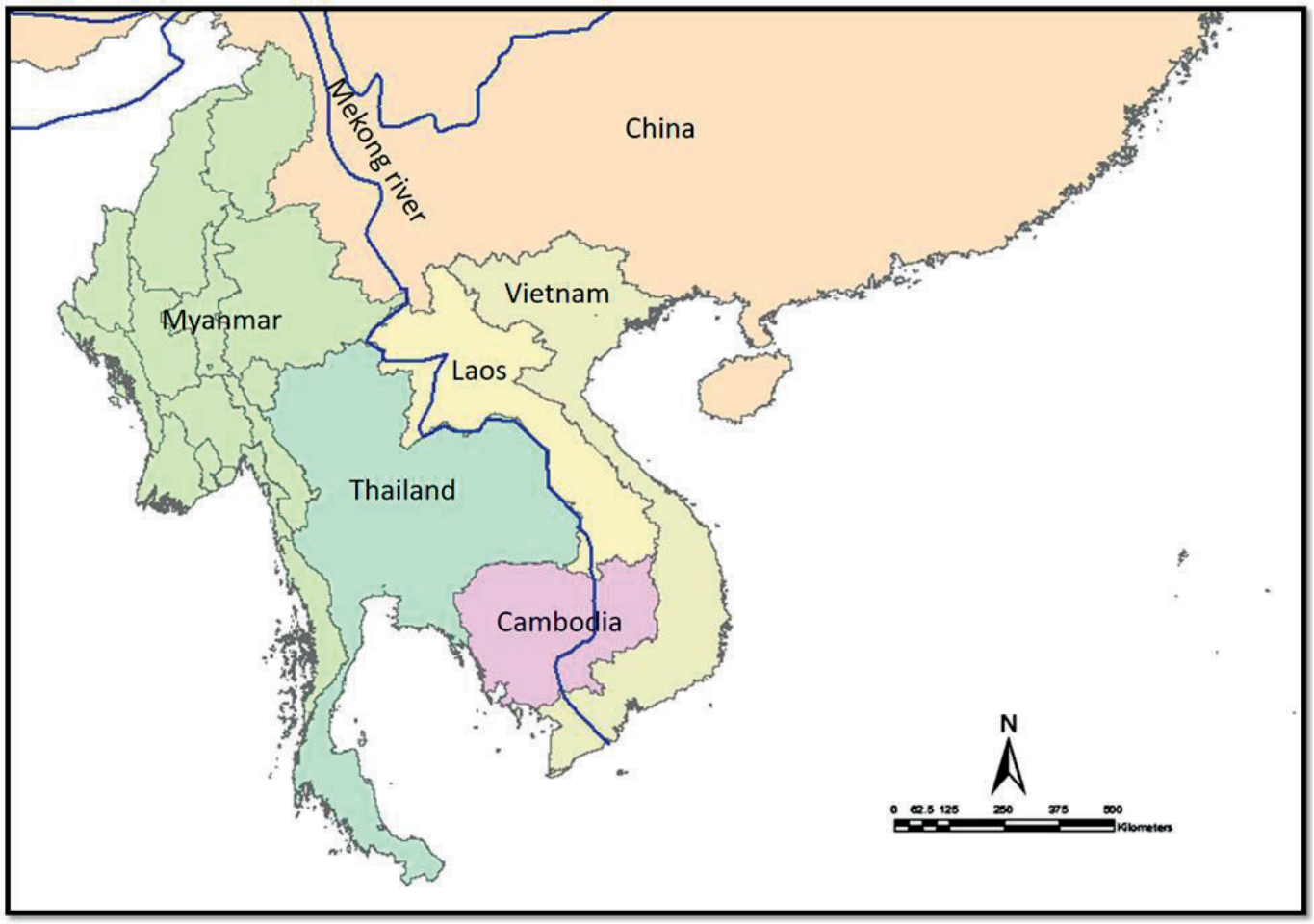

Figure 1. showing regions lower Mekong river basin of Southeast Asia countries created by ArcGIS 10.5.

In southeastern Asia, more than 90 million people are at risk of infection, and at least 10 million people are estimated to be infected by *O. viverrini*. More than ten million people are estimated to be infected with *O. viverrini*: about eight million in Thailand and two million in Lao People's Democratic Republic [26]. The highest prevalence occurs in North and Northeast Thailand, especially in rural populations [27,28,29] and in the adjacent southern and central regions of Lao People's Democratic Republic [30,31]. Human infections with *O. viverrini* and presence of metacercariae in intermediate hosts have been reported in several provinces of Cambodia [32,33,34,35]. Miyamoto et al. (2014) reported *O. viverrini* eggs in human fecal samples from 26 out of 55 surveyed villages in five provinces of Cambodia, among which 15 villages had an egg positive rate >10%. The parasite is also endemic in southern and central parts of Vietnam [25,36]. A survey conducted in 2015 reported that the overall prevalence of *O. viverrini* infection was 11.4% in central Vietnam [25]. The main cause of infection is due to the habit of eating raw or partially cooked meat or fish. When a human consumes raw or semi-cooked fish that may contain metacercariae of the liver fluke and then adult worms inhabit at the bile ducts, where they feed on epithelial cells [26]. Most complications of chronic human opisthorchiasis are hepatobiliary diseases such as hepatomegaly, cholangitis, cholecystitis, peri-ductal fibrosis and gallstones. Recently, an association with cholangiocarcinoma has also been demonstrated [37]. Humans are being infected through ingestion of undercooked or raw freshwater fishes which is contaminated with the infective stage of parasite i.e. metacercariae [10]. Traditional habit of eating raw or undercooked fish is a known risk factor for human trematode infections [12]. Moreover, the infection can also occur via poor personal hygiene and the contamination of food, hands and food preparation utensils that are contaminated with metacercariae. Among the classification of human parasites, trematodes infections are the most important emerging fish-borne parasites. The disease caused by trematode parasite is called trematodiasis. Particularly, small liver flukes, Opisthorchiidae or minute intestinal flukes (MIF), Heterophyidae are most common fish borne trematodes parasite infection in Asia and South East Asia countries [23]. Opisthorchiidae: *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis* are most medically important fish borne parasite in the globe [38]. Heterophyidae, a small-sized fluke, about 1 mm. in length, and is parasitic mostly found in the small intestine. One species of fish can be contaminated with more than one species of fish- borne Trematodes metacercaria. Opisthorchis liver flukes and heterophyid intestinal flukes are highly prevalent fish- borne Trematodes in Southeast Asian countries. Fish-borne trematodiasis caused by liver or minute intestinal flukes (MIF) can cause chronic diseases. Therefore, awareness should be placed on these infections as an important public health issue. This review study aimed to summarize the association among fish species (second intermediate Host) and metacercariae species in lower Mekong basin countries. In addition, this study might provide evidence leading to improved public health awareness for surveillance and control of FBT contamination.

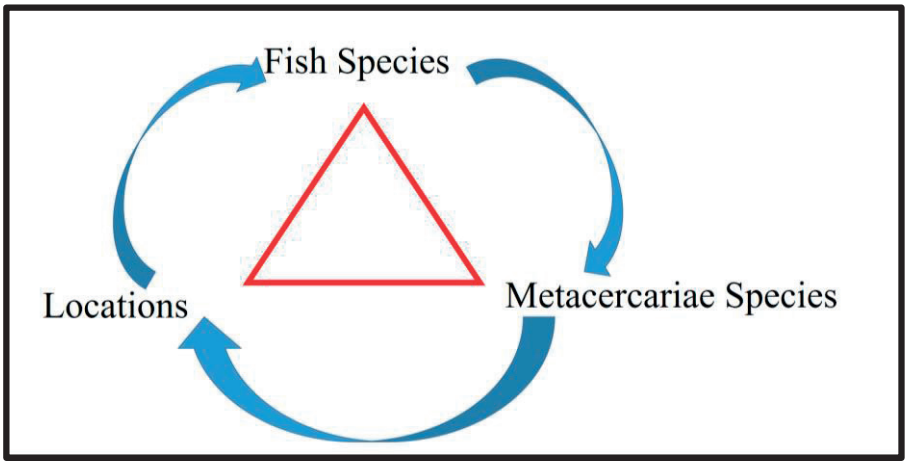

Figure 2. Conceptual frame work of the review about the association of fish species (second intermediate Host) and fish borne trematode metacercariae species.

Species of cyprinoid fishes infected with fish borne trematode metacecariae in lower Mekong basin countries.

No	Location	Species of cyprinoid fishes	Species of metacercariae	References
1.	MaeNgud reservoir, ChiangMai Province	*Thynnichthys thynnoides* *Puntioplites proctozysron* *Hampala macrolepidota* *Puntius leiacanthus* *Puntius gonionotus*	*Haplorchis spp* *H. taichui*	(Sukontason et al 2001)
2.	Southern Cambodia	*Barbodes altus* *Cyclocheilichthys Apagon* *Cyclocheilichthys enoplos* *Hampala dispar* *Hampala macrolepidota* *Henicorhynchus siamensis* *Puntioplites proctozysron* *Puntius Brevis* *Systomus orphoides* *Thynnichthys thynnoides*	*Opisthorchis viverrini*	(Touch et al 2009)
3.	20 provinces in northeastern Thailand	*Cyclocheilichthys armatus, Puntius orphoides,* *Hampala dispar, Henicorhynchus siamensis,* *Osteochilus hasselti, Puntioplites proctozysron*	*Opisthorchis viverrini*	(Pinlaor et al 2013)
4.	Yangon , Myanmar	*Thynnichthys thynnoides* *Chelon macrolepis* *Punitus aurotaeniatus* *Esomus Altus* *Channa striata* *Rhynogobius sp.* *Anabus testudineus* *Trichogaster pectoralis* *Mystacolecucus sp.* *Notopterus notopterus* *Labeo sp.* *Puntioplites proctozysron*	*Haplorchis taichui,* *H. pumilio,* *H.yokogawai, Centrocestus spp.,* *Stellantchasmus falcatus,* *Pygidiopsis cambodiensis,* *Procerovum sp*	(Chai et al 2017)
5.	Khammouane Province, Lao PDR	*Cyclocheilichthys repasson* *Cyclocheilichthys armatus* *Cyclocheilichthys enoplos* *Dangila lineata* *Henicorhynchus lineatus Hampala dispar* *Puntioplites proctzysron Osteochilus waandersii*	*O. viverrini metacercariae*	(Ruenwongsa et al 2009)

List of species of metacercariae that found lower Mekong river basin of Southeast Asia countries .

No	Species of metacercariae	Location , Country	Reference
1	*Opisthorchis viverrini*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al 1976)
	Opisthorchis viverrini	Vientiane Municipality and Savannakhet Province, Lao PDR	(Rim et al 2008)
	Opisthorchis viverrini	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al 2015)
	Opisthorchis viverrini	northeastern of Thailand	(Onsurathum et al. 2016)
	Opisthorchis viverrini	Phnom Penh and Pursat , Cambodia	(Chai et al 2014)
2	*Haplorchis taichui*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al 1991)
	Haplorchis taichui	Vientiane Municipality and Savannakhet Province, Lao PDR	(H. Rim et al 2008)
	Haplorchis taichui	from Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al 2015)
	Haplorchis taichui	Chiang Mai Province, Thailand	(Saenphet et al 2008)
	Haplorchis taichui	Bo Kluea District and Pua District, Nan Province, Thailand	(Dusit et al. 2016)
	Haplorchis taichui	northeastern of Thailand	(Onsurathum et al. 2016)
	Haplorchis taichui,	Yangon, Myanmar	(Chai et al 2017)
3	*Haplorchis pumilio*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al 1991)
	Haplorchis pumilio	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al. 2015)
	Haplorchis pumilio	Yangon, Myanmar	(Chai et al. 2017)
	Haplorchis pumilio,	Phnom Penh and Pursat , Cambodia	(Chai et al. 2014)
4	*Haplorchis yokogawai*	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al. 2015)
	Haplorchis yokogawai	Vientiane Municipality and Savannakhet Province, Lao PDR	(Rim et al 2008)
	Haplorchis yokogawai	Yangon, Myanmar	(Chai et al 2017)
	Haplorchis yokogawai	Phnom Penh and Pursat , Cambodia	(Chai et al 2014)
5	*Haplorchoides mehrai*	Nam Ngum water reservoir in Vientiane Province, Lao PDR	(Scholz et al 1991)
6	*Haplorchoides* sp.	Chiang Mai Province, Thailand	(Saenphet et al 2008)
7	*Centrocestus formosanus*	Vientiane Municipality and Savannakhet Province, Lao PDR	(Rim et al 2008)
	Centrocestus formosanus	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al 2015)
	Centrocestus formosanus	Phnom Penh and Pursat , Cambodia	(Chai et al 2014)
8	*Centrocestus caninus*	Chiang Mai Province, Thailand	(Saenphet et al 2008)
9	*Centrocestus* spp.	Yangon, Myanmar	(Chai et al 2017)
10	*Procerovum varium*	Vientiane Municipality and Champasak Province in Lao PDR	(Eom et al 2015)

11	*Procerovum* sp.	Yangon, Myanmar	(Chai et al 2017)
	Procerovum sp	Phnom Penh and Pursat , Cambodia	(Chai et al 2014)
12	*Pygidiopsis cambodiensis*	Yangon, Myanmar	(Chai et al 2017)
13	*Stellantchasmus falcatus*	Chiang Mai Province , Thailand	(Saenphet et al 2008)
	Stellantchasmus falcatus	Yangon, Myanmar	(Chai et al 2017)

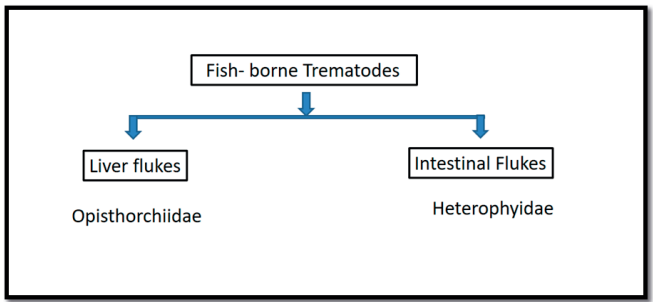

Figure Common Fish- borne Trematodes parasites in lower Mekong basin countries.

Morphologically differential diagnosis of fish borne trematode metacercariae in Southeast Asia countries along the Mekong river basin.

No	Species	Excretory bladder	OS: oral sucker; VS: ventral sucker	Shape	ventro-genital sac and spine	References
1	*Opisthorchis viverrini*	O-shaped excretory bladder	equal sized 2 suckers OS: oral sucker; VS: ventral sucker	elliptical		(Sanpool et al 2018)
2	*Haplorchis taichui*	O-shaped excretory bladder		elliptical	baseball glove-shaped ventro-genital sac with 11-19 chitinous rodlets	(Rim et al 2008)
3	*Haplorchis pumilio*	O-shaped excretory bladder		elliptical	deer horn-like minute spines arranged in 1-2 rows	(Chai et al 2017)
4	*Haplorchoides mehrai*	a rather small excretory bladder		variable in shape,' size		(Scholz et al 1991)
5	*Haplorchis yokogawai*	O-shaped excretory bladder		elliptical or round	U-shaped ventrogenital sac with 70-74 min spines	(Rim et al 2008)
6	*Centrocestus formosanus*	X-shaped excretory bladder	32 circumoral spines around the oral sucker arranged in 2 rows	elliptical	32 circumoral spines	(Chai et al 2017)
7	*Stellantchasmus falcatus*	O-shaped excretory bladder		elliptical	Brownish pigment granules scattered in the body	(Chai et al 2017)
8	*Pygidiopsis cambodiensis*	X shaped excretory bladder.	had an oral sucker ,a pair of eyespots, ventral sucker,	elliptical,	ventrogenital sac,	(Chai et al 2017)
9	*Procerovum sp*	D-shaped (half-moon shaped) excretory bladder with grouped granules.		elliptical	yellow brownish pigment granules scattering in body area of intestinal bifurcation	(Chai et al 2017)

DISCUSSION

Freshwater cyprinid fishes are the second intermediate host of trematode infection such as small liver flukes and minute intestinal flukes. Zoonotic trematode infected fishes are found throughout many parts of lower Mekong basin countries Thailand [39,40,41], Laos [42,43,44, Cambodia [45,46], Vietnam [47,48], Central Region of Myanmar [1,49,50,]. An important public health problem of the spread of fish borne trematodes (FBT) is being highly alarmed the countries of lower Mekong basin [3,5,21,22,23,51,52]. Nevertheless, the contaminated fish species are different in each region. In 2019, *Opisthorchis viverrini* metacercariae were found 4 species of fish forest snakehead (*Channa Lucius*), striped snakehead (*C. striata*), climbing perch (*Anabas testudineus*) and unspecified *Puntioplites sp* [47]. They have reported that Low-Grade Endemicity of Opisthorchiasis, Yangon, Myanmar. They found that *Opisthorchis viverrini* metacercariae in freshwater fish and 0.7% fecal egg-positive rate of residents of *Opisthorchis viverrini* infection [47]. In central Myanmar, Bago region Sanpool et al (2018) reported that *Opisthorchis viverrini* metacercariae were detected in cyprinoid fish, *Puntius brevis.* In Myanmar fish borne trematode infection is first reported in 2017 by Chai et al (2017). Seven species of minute intestinal flukes, i.e., *Haplorchis taichui, H. pumilio, H. yokogawai, Centrocestus* spp*., Stellantchasmus falcatus, Pygidiopsis cambodiensis,* and *Procerovum* sp., were found in fishes from the local market of Yangon, Myanmar. *Haplorchis taichui* metacercariae were infected in 5 species *Thynnichthys thynnoides, Puntius aurotaeniatus, Esomus altus, Mystacoleucus* sp*., Labeo* sp. [50]. Human infections with *O. viverrini* and presence of metacercariae in intermediate hosts have been reported in several provinces of Cambodia [32,33,34,35]. Miyamoto et al reported *O. viverrini* eggs in human fecal samples from 26 out of 55 surveyed villages in five provinces of Cambodia, among which 15 villages had an egg positive rate >10% [33]. The parasite is also endemic in southern and central parts of Vietnam [25,36]. A survey conducted in 2015 reported that the overall prevalence of *O. viverrini* infection was 11.4% in central Vietnam [25].Although not many reported to date from Myanmar, the presence of *O. viverrini* is likely because of its proximity to endemic areas in Thailand and because of an open-borders policy that started in 2015 leading to increasing migration among ASEAN Economic Community (AEC) countries (Thailand, Lao People's Democratic Republic, Cambodia, Vietnam and Myanmar) (ASEAN Economic Community Blueprint. Declaration on the ASEAN Economic Community blueprint 2008. http://asean.org/wp-content/uploads/archive/51 87-10.pdf Accessed 4Mar 2017). Approximately eight million people in Thailand and two million in Lao PDR are estimated to be infected with *O. viverrini* [26] with the high prevalence reported in rural populations of North and Northeast Thailand [12,27,28] and in the adjacent central and southern parts of Lao PDR [30,31]. In Thailand, the epicenter of *O. viverrini* infection is located in the north and northeast Thailand, where high a prevalence of opisthorchiasis coexists with a high incidence of cholangiocarcinoma (CHCA). Southeast Asian liver fluke (*Opisthorchis viverrini*) and Chinese liver fluke (*Clonorchis sinensis*) are classified as Group 1 carcinogens, i.e. they are substantiated and directly cancer-causing agents (Bouvard et al 2009). *O. viverrini* is a food-borne liver fluke that mainly attacks the area of the bile duct. Infection with the parasite, called opisthorchiasis is the major cause of cholangiocarcinoma, a cancer of the bile ducts, in northern Thailand, the Lao People's Democratic Republic, Vietnam and Cambodia [3,21,22,23,50,51,52]. Consumption of raw or inadequately cooked cyprinid fish, as well as related products (often contaminated with *Opisthorchis viverrini and/or Haplorchis taichui*), is one of the major causes of fish-borne trematode infection, which is still endemic in the Greater Mekong Subregion; Vietnam, Laos, Myanmar, Cambodia and Thailand [53]. A chronic infection caused by the Southeast Asian liver fluke (*O. viverrini*) is a critical risk factor for the development of the bile duct cancer cholangiocarcinoma (CCA), which is a major public health concern in Mekong region countries. *O. viverrini* infection in humans occurs via the consumption of a raw or uncooked fish which contains metacercariae. A better understanding of the epidemiology of this fish borne parasites is important to investigate for the prevention of CCA in the community. The review of the fish-borne trematode metacercariae and second intermediate host species could contribute to solving the important public health problem posed by these trematodes and may provide valuable information for prevention and control programs of human liver fluke and intestinal fluke infections for the community.

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