

SPECIAL ARTICLE

Epidemiological Status of *Opisthorchis viverrini* Metacercariae in Freshwater Fish Across the Greater Mekong Subregion

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Received 25 May 2026

Revised 7 June 2026

Accepted 8 June 2026

J Med Urban Health

2026;70(3):e7962

<https://doi.org/10.62691/jmuh.2026.7962>

ABSTRACT

Opisthorchis viverrini (a Group 1 biological carcinogen) remains a major public health problem in the Greater Mekong Subregion because of its strong association with cholangiocarcinoma. Given that human infection mainly occurs through the consumption of raw or undercooked cyprinoid fish containing infective metacercariae. This review summarizes the epidemiological status, geographical distribution, host specificity, and transmission dynamics of *O. viverrini* metacercariae in cyprinoid fish across Thailand, Lao People's Democratic Republic (Lao PDR), Cambodia, Myanmar, and Vietnam. The reviewed studies demonstrated considerable geographical variation in *O. viverrini* metacercariae prevalence throughout the Greater Mekong Subregion, with particularly high endemicity reported in Cambodia and Lao PDR, especially within Mekong River-associated ecosystems. In Cambodia, prevalence reached 90.2% in cyprinoid fish from Kandal Province, whereas substantially lower prevalence levels were generally observed in inland regions of Thailand. Species-specific variation was also evident, with *Cyclocheilichthys* spp., *Hampala dispar*, and *Systomus orphoides* identified as important second intermediate hosts in the transmission cycle of *O. viverrini*. Seasonal fluctuations, aquatic ecology, environmental contamination, and inadequate sanitation were consistently associated with transmission intensity. The available evidence indicates that *O. viverrini* metacercariae remain widely distributed in freshwater ecosystems across the region, reflecting the persistent risk of fish-borne transmission in endemic communities. Strengthened surveillance of high-risk cyprinoid fish species, improved sanitation, and sustained behavioral interventions remain essential for reducing transmission and the long-term burden of cholangiocarcinoma in the region.

Keywords: cyprinoid fish, Greater Mekong Subregion, metacercariae, One Health, *Opisthorchis viverrini*

INTRODUCTION

Opisthorchiasis caused by *Opisthorchis viverrini* remains a major food-borne trematode infection in the Greater Mekong Subregion (GMS), particularly in Thailand, Lao People's Democratic Republic (Lao PDR), Cambodia, Myanmar, and Vietnam.¹ Given that *O. viverrini* has been classified as a Group 1 biological carcinogen by the International Agency for Research on Cancer, chronic opisthorchiasis is closely linked to hepatobiliary diseases, especially cholangiocarcinoma (CCA).² The highest endemicity occurs in Northeastern Thailand and Lao PDR, where transmission is strongly associated with the traditional consumption of raw or undercooked freshwater fish dishes, including *koi-pla*, *pla-som*, and *pla-ra*.³

The life cycle of *O. viverrini* involves freshwater *Bithynia* snails as first intermediate hosts and cyprinoid fish as second intermediate hosts carrying infective metacercariae.⁴ Although human infection mainly occurs through the consumption of raw or undercooked cyprinoid fish containing infective metacercariae, viable metacercariae have also been detected in traditional fish products.³ Research has shown marked geographical variation in metacercarial prevalence among cyprinoid fish across the GMS, particularly in Mekong floodplain ecosystems.⁵ High metacercarial prevalence in cyprinoid fish has been reported in Cambodia and Lao PDR, whereas lower but persistent fish-borne endemicity has been observed in several regions of Thailand.^{5,6} Therefore, the surveillance of metacercarial infection in cyprinoid fish remains essential for epidemiological monitoring and disease prevention.⁷ This review summarizes current evidence regarding the prevalence, geographical distribution, host specificity, and transmission dynamics of *O. viverrini* metacercariae in cyprinoid fish throughout the GMS. A summary of the epidemiological studies included in this review is presented in **Table 1**.

CONTENT OF REVIEW

1. Regional Epidemiology of *O. viverrini* Metacercariae

The prevalence of *O. viverrini* metacercariae markedly varies across the GMS, with major endemic hotspots being reported in Thailand, Lao PDR, Cambodia, Myanmar, and Vietnam. Freshwater cyprinoid fish serve as the principal second intermediate hosts sustaining transmission to humans. The prevalence and intensity of infection reported in previous studies are summarized in **Table 2**.

1.1 Thailand

Thailand, particularly the northeastern region, is recognized as a major endemic focus of opisthorchiasis and CCA. According to large-scale surveys across 20 northeastern

provinces, *O. viverrini* metacercariae have been detected in 24.3% of sampling sites, with high infection intensities being reported in the provinces of Amnat Charoen, Nakhon Phanom, Mukdahan, Khon Kaen, and Si Sa Ket.⁸ In Udon Thani Province, the metacercarial prevalence in fish from the Huay Luang Reservoir ranged from 21.6% to 22.8%, whereas surveys in the Nong Han Wetland reported an overall prevalence of 15.1% among 11 cyprinoid fish species.^{4,9} Similarly, surveillance in Nakhon Ratchasima Province demonstrated an overall prevalence of 12.3%; however, some districts showed markedly higher infection rates reaching 78.1%.⁷ In Nakhon Phanom Province, the infection intensity varied geographically, with *Hampala dispar* harboring the highest parasite burden.¹⁰ Transmission is further facilitated through local fish trade networks, where infected fish are widely distributed through regional markets.¹¹

1.2 Lao PDR

Lao PDR remains one of the most highly endemic areas for *O. viverrini* metacercarial infection in the GMS. According to surveys in Vientiane Municipality, the overall fish infection prevalence was 49.5%, with *Cyclocheilichthys apogon* and *C. armatus* showing infection rates of up to 100% in some samples.¹² High prevalence has also been documented in Khammouane Province, particularly within the NamDon, NamPakane, and Mekong River systems, where infection rates reached 51.9% in Thakhek district.⁵ Similarly, studies from Savannakhet and Saravane provinces reported prevalence rates ranging from 38.5% to 42.1%.¹³ Based on these findings, there is widespread and intense transmission in Lao PDR, which is likely associated with the traditional consumption of raw-fish and persistent environmental contamination.^{12,14}

1.3 Cambodia

Cambodia exhibits highly localized transmission of *O. viverrini* metacercariae, particularly along the Mekong River and Tonle Sap Lake systems.⁶ Kandal Province represents a major endemic hotspot, where an exceptionally high prevalence of 90.2% was reported among freshwater fish, with *Systemus orphoides* and *Barbonymus altus* showing infection rates of up to 100%.⁶ In Takeo Province, the metacercarial prevalence reached 38.1%, whereas surveys of market fish in Phnom Penh reported infection rates ranging from 23.7% to 50.0%, indicating widespread distribution through local trade networks.^{6,15} These findings highlight substantial regional variation in endemicity and underscore the importance of freshwater ecosystems, fish trade, and traditional fish consumption practices in sustaining transmission.⁶

Table 1 Summary of Study Characteristics Investigating the Prevalence and Epidemiology of *Opisthorchis viverrini* Metacercariae in the Greater Mekong Subregion

Author	Year	Country	Study Area	Number of Fish Examined	Main Findings
Rim et al.	2008	Lao PDR	Vientiane & Savannakhet	Not specified	High endemicity in Lao PDR, with a 49.5% prevalence in fish from Vientiane Municipality.
Kaewpitoon et al.	2012	Thailand	Nakhon Ratchasima	640 fish	12.3% overall prevalence in fish, with hotspots reaching 78.1% in Sida and KiaKham Thale So districts.
Rim et al.	2013	Lao PDR	3 Provinces	Not specified	Fish infection rate of 14.5% across Luang Prabang, Khammouane, and Saravane.
Prakobwong et al.	2017	Thailand	Udon Thani Province	1,831 fish	Prevalence reached 22.8% near Huay Luang River.
Prakobwong et al.	2017	Thailand	Huay Luang Dam	2,189 fish	Prevalence was 16.9%, intensity in fish peaks during the dry season (January-April).
Phyo Myint et al.	2020	Myanmar	Tachileik (Mekong)	689 fish	Confirmed the first discovery of carcinogenic OvMc in the Mekong region of Myanmar.
Kaensa et al.	2025	Thailand	Nong Han Wetland	392 fish	Average OvMc prevalence in cyprinoid fish was 13.9% across multiple sampling sites.
Prakobwong et al.	2025	Thailand	Udon Thani Province	420 fish	A One Health approach significantly reduced prevalence from 21.91% to 6.0%.
Charoensuk et al.	2026	Thailand	Huay Luang River	1,377 fish	8.5% prevalence; established fishborne trematodes as bioindicators for heavy-metal contamination.

Abbreviation: OvMc, *O. viverrini* metacercariae

Table 2 Prevalence and Intensity of *Opisthorchis viverrini* Metacercariae in Cyprinoid Fish Across the Greater Mekong Subregion

Country	Province/Area	Fish Species	Prevalence (%)	Number of Infected Fish (n)	Number of Examined (n)	Mean Intensity (OvMc/fish)
Thailand	Udon Thani	<i>Henicorhynchus siamensis</i>	27.7%	186	671	17.6
	Udon Thani	<i>Cyclocheilichthys repasson</i>	21.9%	164	748	14.7
	Nakhon Ratchasima	<i>Cyclocheilichthys armatus</i>	19.16%	7	140	ND
	Nakhon Ratchasima	<i>Cyclocheilichthys repasson</i>	18.07%	23	126	ND
	Nong Han Wetland	<i>Cyclocheilichthys apogon</i>	20.3%	37	182	3.7
	Nong Han Wetland	<i>Anematichthys repasson</i>	17.8%	8	45	5.8
	Upper northeast	<i>Mystacoleucus obtusirostris</i>	52.94%	9	17	ND
	Upper northeast	<i>Osteochilus melanopleura</i>	43.47%	10	23	ND

Table 2 Prevalence and Intensity of *Opisthorchis viverrini* Metacercariae in Cyprinoid Fish Across the Greater Mekong Subregion (cont.)

Country	Province/Area	Fish Species	Prevalence (%)	Number of Infected Fish (n)	Number of Examined (n)	Mean Intensity (OvMc/fish)
Lao PDR	Vientiane Municipality	<i>Cyclocheilichthys apogon</i>	100%	15	15	304.1
	Vientiane Municipality	<i>Cyclocheilichthys armatus</i>	100%	20	20	145.1
	Savannakhet	<i>Puntius brevis</i>	92.0%	23	25	360.0
	Savannakhet	<i>Puntioplites falcifer</i>	33.3%	3	9	1.3
	Khammouane	<i>Dangila lineata</i>	69.6%	64	92	2.6 per gram
	Khammouane	<i>Cyclocheilichthys repasson</i>	58.5%	117	195	2.63 per gram
Cambodia	Kandal Province	<i>Systemus orphoides</i>	100%	17	17	37.7
	Kandal Province	<i>Barbonymus altus</i>	100%	14	14	21.6
	Takeo Province	<i>Osteochilus schlegelii</i>	100%	3	3	34.7
	Phnom Penh	<i>Barbonymus schwanefeldi</i>	100%	5	5	74.6
Myanmar	Tachileik (Mekong)	<i>Cyclocheilichthys repasson</i>	2.64%	4	151	2.75
	Yangon City	<i>Puntioplites</i> sp.	10.0%	1	10	1.0
Vietnam	Phu Yen Province	<i>Carassius auratus</i>	28.1%	ND	ND	28.3

Abbreviation: n, number; ND, not determined in the specific study table or text provided; OvMc, *O. viverrini* metacercariae

1.4 Myanmar

In Myanmar, *O. viverrini* metacercariae distribution across several regions remains persistent but at a relatively low endemic level.¹⁶ In Tachileik, which is located within the Mekong River Basin, *O. viverrini* metacercariae were detected in *Cyclocheilichthys repasson* with a prevalence of 2.64% and a mean intensity of 2.75 cysts per infected fish, representing the first documented occurrence of carcinogenic liver fluke metacercariae in this part of Myanmar.¹⁶ Nevertheless, several non-cyprinoid fish species have occasionally been reported to harbor *Opisthorchis*-like metacercariae, although their epidemiological importance remains considerably lower and less clearly defined than that of cyprinoid hosts. In addition, surveys in Yangon City reported *O. viverrini* metacercariae in 2.9% of fish that were examined, including *Anabas testudineus* and *Channa striata*, indicating that low-level transmission may persist within certain urban and peri-urban freshwater ecosystems. However, the contribution of non-cyprinoid fish species to regional transmission dynamics remains uncertain because morphological identification alone may be insufficient to confirm species-level classification of

metacercariae. Consequently, additional molecular and epidemiological investigations are required to clarify the transmission significance of these alternative fish hosts.¹⁷

1.5 Vietnam

In Vietnam, human liver flukes show distinct geographical distribution patterns, with *Clonorchis sinensis* predominating in the northern provinces and *O. viverrini* being mainly endemic in the central and southern regions.¹⁸ This pattern aligns with the broader regional distribution of these parasites within the Lower Mekong Basin. In Central Vietnam, Phu Yen Province has been identified as a critical “hotspot” for opisthorchiasis.¹⁸ Furthermore, according to surveillance of intermediate hosts in this province, crucian carp (*Carassius auratus*) harbor a significant parasite burden, with metacercarial prevalence reaching 28.1% and a mean intensity of 28.3 cysts per fish.¹⁹ In Southern Vietnam, *O. viverrini* metacercariae have been detected in snakehead fish (*Channa striata*) from An Giang Province, albeit at a low prevalence of 1.9%.¹⁸ However, the definitive identification of

O. viverrini in non-cyprinid hosts, including snakeheads, remains challenging. Moreover, early reports often relied on morphological criteria that may overlap with other *Opisthorchis*-like flukes or related species such as *O. lobatus*.^{18,19} Consequently, molecular confirmation and experimental infections are frequently needed to clarify the taxonomic status of these isolates and determine the potential role of non-cyprinoid as viable intermediate hosts.

2. Host Specificity and Transmission Dynamics

O. viverrini transmission involves a broad range of cyprinoid fish hosts reported to harbor infective metacercariae.¹⁸ However, members of the Cyprinidae family are the most epidemiologically important. Several species consistently show high infection burdens, including *Cyclocheilichthys armatus*, *C. apogon*, *Hampala dispar*, *Henicorhynchus siamensis*, *Puntioplites proctozysron*, and *Puntius brevis*, often with high prevalence and intensity.^{7,8,12} In highly endemic areas (e.g., Vientiane Municipality and Savannakhet Province), infection in *C. armatus* and *C. apogon* has reached 100%, with very high cyst burdens per fish.^{12,14} Nevertheless, susceptibility varies markedly among species within the same ecosystem. For example, *Mystacoleucus obtusirostris* has shown high prevalence (52.94%), whereas *Barbonymus gonionotus* exhibits much lower infection rates (1.71%), reflecting species-specific differences in innate susceptibility.²⁰

3. Environmental and Ecological Factors Influencing Transmission

3.1 Seasonal Variation

The transmission dynamics of *O. viverrini* demonstrate marked seasonal variation, with metacercarial prevalence and intensity in cyprinoid fish generally peaking during the dry and cool-dry seasons (November to April).^{4,9} During this period, reduced water levels concentrate snails, fish, and parasites within smaller aquatic habitats, increasing host density and enhancing contact between cercariae and susceptible fish hosts.^{5,9,10} In addition, temperature strongly influences transmission, as warmer conditions (around 34°C) promote *Bithynia* snail infection and cercarial shedding, whereas temperatures exceeding 35°C reduce egg and metacercarial viability.^{9,10} Conversely, the rainy season is associated with increased water volume and flow, which dilute parasite stages and lower infection prevalence in fish despite runoff-associated fecal contamination.⁹ The major environmental, ecological, and behavioral determinants of *O. viverrini* transmission are summarized in **Table 3**.

3.2 Aquatic Ecology and Hydrological Connectivity

Aquatic habitat configuration plays a major role in the transmission dynamics of *O. viverrini*. Lentic environments (e.g., dams and reservoirs) generally exhibit substantially higher metacercarial prevalence than lotic systems, with infection rates reaching 70% in some areas; meanwhile, fast-flowing rivers in Northeastern Thailand showed much lower prevalence rates of approximately 7.7%.⁸ Slow-moving or stagnant waters promote organic matter accumulation and provide favorable ecological conditions for *Bithynia* snails and cyprinoid fish, thereby increasing opportunities for cercarial transmission.⁹ Furthermore, anthropogenic modifications, including irrigation canal construction, may expand transmission hotspots by increasing snail habitats.²¹ By contrast, lotic systems are characterized by stronger water flow that dilutes parasite eggs and cercariae, thereby reducing the transmission efficiency.⁹

3.3 Environmental Contamination

Inadequate waste management is a major driver of sustained *O. viverrini* transmission in freshwater ecosystems. In the Nong Han Wetland of Northeastern Thailand, fecal coliform levels were strongly positively correlated with *O. viverrini* metacercariae prevalence in fish ($r = 0.981$, $P \leq 0.001$), highlighting the role of human waste contamination in creating transmission hotspots.⁹ Open defecation and untreated wastewater discharge introduce large numbers of parasite eggs into aquatic environments, where they are consumed by *Bithynia* snails.^{8,21} Given that natural snail infection rates are typically below 1%, continuous environmental contamination is necessary to sustain transmission and metacercarial infection in fish. Consequently, proximity to contaminated water sources is a significant risk factor for infection in nearby human populations.²²

3.4 Pollution and Heavy Metals

According to recent toxicological research, fish-borne trematode metacercarial infections have been identified as robust, site-specific bioindicators for heavy-metal pollution in freshwater environments. Investigations in Udon Thani Province have determined that cadmium is the strongest predictor of *O. viverrini* metacercariae infection intensity. According to multivariate regression, each 1-ppm increase in cadmium concentration corresponds to an average rise of 5.36 metacercariae/infected fish.²³ Heavy-metal contamination, often derived from community waste (e.g., nickel-cadmium batteries), induces physiological and oxidative stress, which impairs the fish host's immune competence, thereby increasing its vulnerability to parasite establishment.

Table 3 Major environmental, ecological, and behavioral factors associated with *Opisthorchis viverrini* transmission

Factor	Mechanism	Transmission Significance	Supporting Studies
Behavioral: Raw fish consumption	Ingestion of viable metacercariae (OvMc) in traditional dishes such as <i>koi-pla</i> (raw salad), <i>pla-som</i> (short-term fermented), and <i>lab-pla</i> .	Primary driver of human infection; positive correlation between frequency of consumption and infection prevalence.	Prakobwong et al. (2017)
Behavioral: Poor sanitation	Open defecation or the use of unhygienic latrines near water bodies discharges eggs into the environment.	Facilitates the human-to-snail linkage; high fecal coliform levels are strongly correlated ($r = 0.981$) with OvMc prevalence.	Kaewpitoon et al. (2012); Kaensa et al. (2025); Prakobwong et al. (2025)
Ecological: Host feeding behaviors	Certain cyprinids (<i>Hampala dispar</i> , <i>Barbonymus gonionotus</i>) forage in shallow, vegetated shorelines where <i>Bithynia</i> snails and fecal contamination aggregate.	Results in high infection intensity burdens in specific "target" fish species compared to others.	Kaensa et al. (2025); Namsanor et al. (2020)
Ecological: Reservoir host roles	Domestic cats and dogs scavenge on raw fish scraps or discarded rotten fish near reservoirs, shedding eggs into water sources.	Sustains environmental contamination and reinfection cycles even when human cases are treated.	Prakobwong et al. (2017)
Environmental: Seasonality	Receding water levels in the dry season concentrate snails, fish, and parasites, while higher temperatures can boost snail infection rates.	Significant peak in metacercarial prevalence and intensity in fish during the dry season (November–April).	Kaensa et al. (2025)
Environmental: Habitat type	Lentic water bodies (dams/reservoirs) favor organic matter accumulation and slow water flow, conditions that facilitate parasite persistence.	Fish from dams/reservoirs exhibit significantly higher prevalence (up to 70%) than those from lotic river systems (7.7%).	Pinlaor et al. (2013); Prakobwong et al. (2017)
Environmental: Heavy metal pollution	Cadmium concentrations (from community waste like batteries) overlap with high parasite intensity hotspots.	Cadmium was positively associated with higher infection intensity in endemic freshwater ecosystems.	Charoensuk et al. (2026)
Socio-cultural: Gender socialization	In rural communities, consumption of raw fish with alcohol is often associated with traditional "masculine" behavior.	Significant sex disparity; males show 2–4 times greater odds of infection than females across most age groups.	Buathong et al. (2024)
Commercial: Fish trade networks	Improvement in transportation allows parasitized fish to be sold in markets up to 100 km away from their point of capture.	Dispersion of the parasite into non-endemic areas or areas where transmission was previously controlled.	Charoensuk et al. (2022)

Abbreviation: OvMc, *O. viverrini* metacercariae

From a One Health perspective, the co-occurrence of bioaccumulated toxic metals and carcinogenic flukes in commonly consumed fish presents a synergistic public health hazard that may exacerbate the risk of cholangiocarcinoma.²³

4. Sociobehavioral and Economic Drivers

Human infection with *O. viverrini* is primarily associated with the traditional consumption of raw or undercooked

freshwater fish dishes, which may contain viable metacercariae; these parasites are effectively destroyed by prolonged salt fermentation and thorough cooking.³ Domestic animals, especially free-roaming cats, also serve as important reservoir hosts, with reported infection rates ranging from 30% to 53.1%, thereby sustaining environmental contamination and parasite transmission.²⁴ Moreover, higher infection prevalence has been observed among males and individuals aged

40–60 years, likely associated with occupational exposure, traditional dietary practices, and cumulative long-term consumption of infected fish.⁴

5. Public Health Significance and One Health Implications

The current control strategies for opisthorchiasis are increasingly adopting a One Health approach that integrates human, animal, and environmental interventions to interrupt transmission.²⁴ In Thailand, the Lawa Model combines community health education, school-based programs, training of health volunteers, and biological snail control using ducks to reduce *Bithynia* snail populations.²⁵ Similar interventions around the Huay Luang Reservoir in Udon Thani Province significantly reduced human infection prevalence, reservoir host infections, and metacercarial prevalence in cyprinoid fish from 14.1% to 0.9%, 21.3% to 3.8%, and 21.9% to 2.2% over six years, respectively.²⁴ These reductions were largely associated with behavioral changes and decreased consumption of raw fish following targeted health education campaigns.²⁴ Nevertheless, long-term elimination remains challenging because of the persistent cultural practice of consuming raw fish, which leads to frequent reinfection after treatment.²⁴

CONCLUSION

Opisthorchiasis remains a major public health challenge in the GMS because of persistent environmental contamination, high prevalence of infective metacercariae in freshwater fish, and culturally embedded practices of consuming raw fish. Although health education and control programs have successfully reduced infection rates in some endemic communities, sustainable elimination remains difficult because reinfection frequently occurs. Therefore, the long-term control of *O. viverrini* requires continuous surveillance, improved sanitation, behavioral change interventions, and integrated One Health strategies targeting humans, reservoir hosts, and the aquatic environment to effectively reduce transmission and the associated burden of cholangiocarcinoma.

Conflict of Interest

The author declares no conflicts of interest.

Acknowledgments

The author would like to acknowledge the financial support provided by the Faculty of Medicine Vajira Hospital, Navamindradhiraj University, for English language editing services.

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