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Opisthorchis viverrini Metacercariae in Cyprinoid Fish from Three Rivers in Khammouane Province, Lao PDR

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Abstract

This study aimed to determine the prevalence, intensity and distribution of *Opisthorchis viverrini* metacercarial infection in cyprinoid fish collected from three rivers in Khammouane Province, central Lao PDR, between April 2007 and February 2008. A total of 1,356 freshwater fish were collected; of these, 1,246 cyprinoid fish of 18 species were examined for *O. viverrini* metacercariae–only 8 species of cyprinoid fish were infected with *O. viverrini* metacercariae. Infection rates were as follows: *Cyclocheilichthys repasson* (58.5%), *C. armatus* (43.1%), *C. enoplos* (10.0%), *Dangila lineata* (69.6%), *Henicorhynchus lineatus* (42.9%), *Hampala dispar* (44.4%), *Puntioplites proctzysron* (26.8%), *Osteochilus waandersii* (30.5%); and the mean ± SD intensities of infection were 2.63 ± 1.11, 1.05 ± 0.53, 0.90 ± 0.43, 2.60 ± 0.98, 2.4 ± 0.98, 0.92 ± 0.53, 0.93 ± 0.48, and 1.90 ± 0.45, respectively. The prevalence of *O. viverrini* metacercarial infection among cyprinoid fish from Namdone and Napakane increased markedly during the dry season. *O. viverrini* metacercariae were found scattered throughout the entire body; however, the body muscles harbored the highest density of metacercariae, followed by the fins, head, and visceral organs, respectively.

Keywords: *Opisthorchis viverrini*, metacercariae, cyprinoid fish, prevalence, intensity, distribution

Introduction

Lao PDR is a landlocked nation in central Southeast Asia, which shares borders with 5 countries–China, Myanmar, Vietnam, Thailand, and Cambodia. The total population is slightly over 5 million. The population density, compared with its neighbours, is low. A quarter of Lao territory is located in the Mekong River Basin. The Mekong River runs through the whole country, from north to south, in a south-easterly direction.

Fish-borne trematode infections are a serious public-health problem [1]. Fish-borne zoonotic parasites, liver and intestinal flukes, are recognized as an important group of emerging and re-emerging human pathogens [2,3]. In Lao PDR, opisthorchiasis, or liver-fluke infection, is a major public health problem, and it is estimated that > 2 million people are infected with the causative parasite, *Opisthorchis viverrini* [2].

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O. viverrini was first diagnosed in 1929 by Bedier and Chesneau, in Thakek (Khammouane Province) and Vientiane, where infection rates were 23 and 15%, respectively [4,5]. In 2000, Kobayashi conducted an epidemiological survey of opisthorchiasis in Khammouane Province, Lao PDR, and found the prevalence still very high, at about 60% [6]. A nationwide survey in 18 administrative districts also reported a high prevalence of opisthorchiasis among primary-school children, particularly in central and southern Laos, along the Mekong River [7]. This parasite requires two kinds of intermediate hosts, a freshwater snail and a freshwater fish, to complete its life cycle. Humans acquire infection by eating raw or undercooked freshwater fish containing the infective stage, or metacercaria, of *O. viverrini*. Cyprinoid fish have been reported as an important intermediate host of *O. viverrini* in Vientiane and Saravane, Lao PDR [8,9].

However, several aspects of *O. viverrini* infection have never been studied in Khammouane Province. The source of infection, the infection status of *O. viverrini* metacercariae in freshwater fish, especially cyprinoids, have not yet been elucidated. The present study aimed to determine the prevalence, intensity and distribution of *O. viverrini* metacercariae in cyprinoid fish collected from three rivers in Khammouane Province.

Materials and methods

Fish were collected from 3 rivers–the Namdone, Nampakane, and Mekong–in Khammouane Province (Fig 1), about 35 km from the town of Thakek. Cyprinoid fish commonly eaten raw by the people of Khammouane Province were purchased and collected from local markets near the river between April 2007 and March 2008. Dead fish were kept in labelled plastic bags and transported in an ice box to the laboratory at the

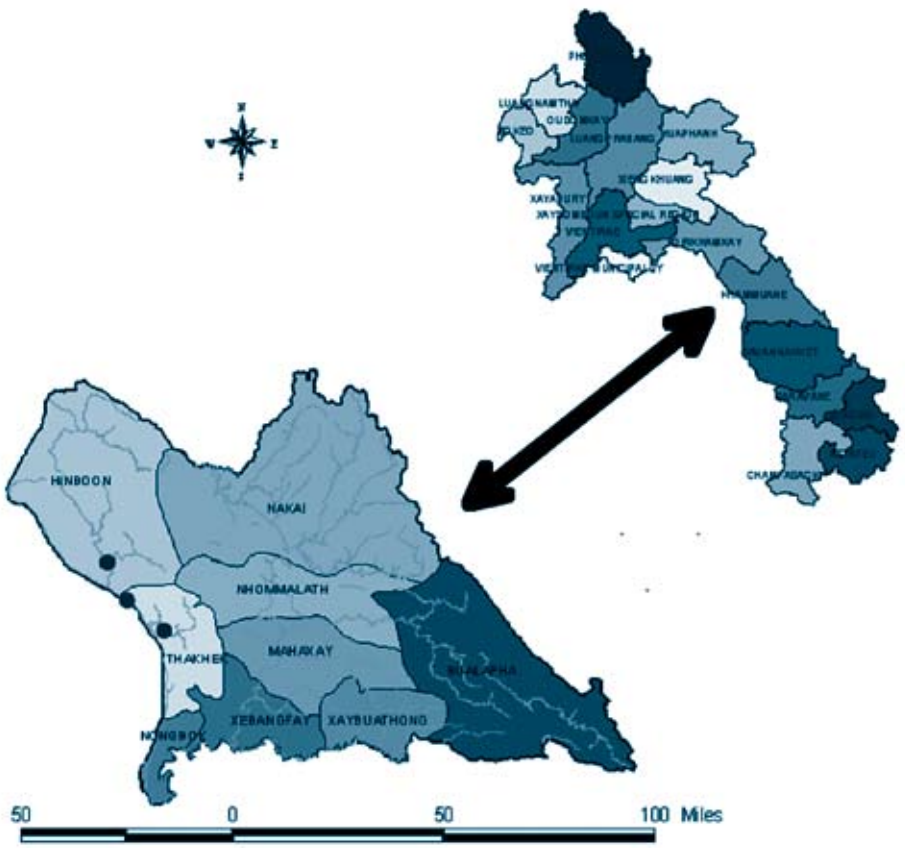

Fig 1 Administrative map of Lao PDR and the study sites in Khammouane Province.

Department of Helminthology, Bangkok, within a week after collection.

The fish were separated and grouped according to their morphological characteristics. For each group, fish samples were photographed and kept in a preserved state with a label, for further identification to species; the rest were used in the study.

After thorough washing under tap water, all fish were measured and weighed, then inspected for metacercariae using the standard pepsin digestion method described by the WHO, 1995. The fish were chopped finely, digested with 1% acid-pepsin, and incubated for 2-3 hours at 37°C, then strained and processed by sedimentation method. Using the morphological criteria of Kaewkes (2003), all metacercariae obtained were separated and grouped [10]. Metacercariae with characteristics similar to *O. viverrini* were counted and recorded. Some of these were inoculated into a hamster, the experimentally definitive host of *O. viverrini*, to confirm species identification.

The fish were divided into fins, muscles, head, and viscera. Each organ was inspected for *O. viverrini* metacercariae using the above-mentioned technique.

Results

A total of 1,356 fish of 24 species in 6 families were collected from three different study sites, and investigated for *O. viverrini* metacercariae. Eighteen species of 1,246 fish were in the family Cyprinidae. The total numbers of cyprinoid fish collected from the Namdone, Nampakane, and Mekong rivers were 569, 441, and 236, respectively (Table 1). Eight species of cyprinoid fish collected from the 3 rivers were infected with *O. viverrini* metacercariae; the infection rate was 44.1% (369/836).

In the Namdone and Nampakane rivers, an *O. viverrini* metacercarial infection rate of > 50% was found predominantly among cyprinoid fish, *ie*, *Dangil lineata*, *Cyclocheilichthys repasson,* and *Hampala dispar*, while in the Mekong, *Puntioplites proctzysron* was predominated (18%). Three species of cyprinoid fish found in three rivers–*C. repasson*, *Henicorhynchus lineatus,* and *P. proctzysron*–were found to harbor *O. viverrini* metacercariae (Table 2).

The prevalence of *O. viverrini* metacercarial infection among cyprinoid fish decreased during the rainy season, with 34.8% in the Namdone, 35% in the Nampakane, and 9.5% in the Mekong. It increased markedly during the dry season (November to April), with 67.6% in the Namdone, 70.3% in the Nampakane, but a relatively small change in the Mekong (Fig 2).

The mean intensities of *O. viverrini* metacercarial infection per gram of cyprinoid fish collected from the Namdone, Nampakane and Mekong rivers were 2.33 ± 1.12, 2.18 ± 1.16, and 1.03 ± 1.15, respectively. The mean number of *O. viverrini* metacercariae infection per gram of fish per species is shown in Table 3. *O. viverrini*

Table 1 Fish collected at the 3 study sites, 2007-2008.

Fish collected	Weight (g) min-max	Length (cm) min-max	Total no. of fish collected (%)	No. of fish collected per site (%)		
				Namdone	Nampakane	Mekong
Cyprinoid	7.5-19.5	4.5-25.5	1,246 (91.88)	569 (94.21)	441 (91.31)	236 (87.73)
Non-cyprinoid	4.5-20.5	4.5-40.5	110 (8.12)	35 (5.79)	42 (8.69)	33 (12.27)
Total	4.5-20.5	4.5-40.5	1,356 (100)	604 (100)	483 (100)	269 (100)

Table 2 *O. viverrini* metacercarial infections among cyprinoid fish at the 3 study sites.

No.	Fish species	Number of fish infected / examined (%)			
		Namdone	Nampakane	Mekong	Total
1	*Cyclocheilichthys repasson* (Pa-kawmesadeng)	60/97 (62)	55/81 (64)	2/17 (11.7)	117/195 (58.5)
2	*Cyclocheilichthys armatus* (Pa-kawna)	19/44 (43)	12/28 (43)	NA	31/72 (43.1)
3	*Cyclocheilichthys enoplos* (Pa-chakok)	2/15 (13)	4/13 (31)	0/32 (0)	6/60 (10.0)
4	*Dangila lineata* (Pa-kulum)	33/42 (79)	35/42 (83.3)	0/8 (0)	64/92 (69.6)
5	*Henicorhynchus lineatus* (Pa-lankong)	41/90 (46)	33/70 (47)	2/17 (12)	76/177 (42.9)
6	*Hampala dispar* (Pa-suot)	6/11 (55)	10/20 (50)	0/9 (0)	16/36 (44.4)
7	*Puntioplites proctzysron* (Pa-marman)	20/56 (35.6)	11/39 (28)	13/69 (18)	44/164 (26.8)
8	*Osteochilus waandersii* (Pa-chewkoun)	7/19 (36.8)	4/10 (40)	0/7 (0)	11/36 (30.5)
	Total	188/374 (50.37)	164/303 (54.13)	17/159 (10.79)	369/836 (44.1)

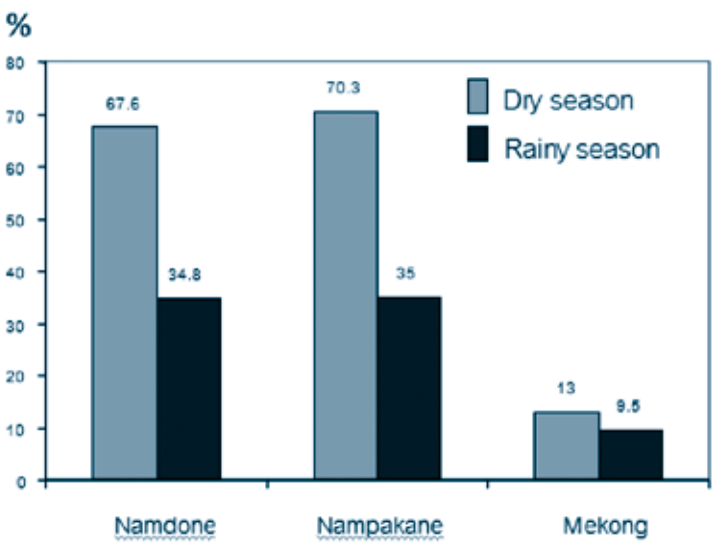

Fig 2 Comparison of the prevalence of *O. viverrini* metacercarial infection in cyprinoid fish, between dry and rainy seasons, at the 3 study sites.

metacercariae were found widely distributed in the muscles of the fish, followed by the fins, head, and visceral organs (Fig 3).

Discussion

The results of the present study are important from both parasitological and public-health viewpoints. Opisthorchiasis remains an important problem in Lao PDR. The areas surrounding the 3 rivers in Khammoune Province that were selected for this study are endemic for trematode infections. Examination of freshwater fish collected from the Namdone, Nampakane, and Mekong Rivers, revealed the presence of *O. viverrini* metacercariae in freshwater fish for the first time in Khammouane Province, in central Laos. In Savannakhet, Saravane, and Vientiane, *O. viverrini* metacercariae have been found in

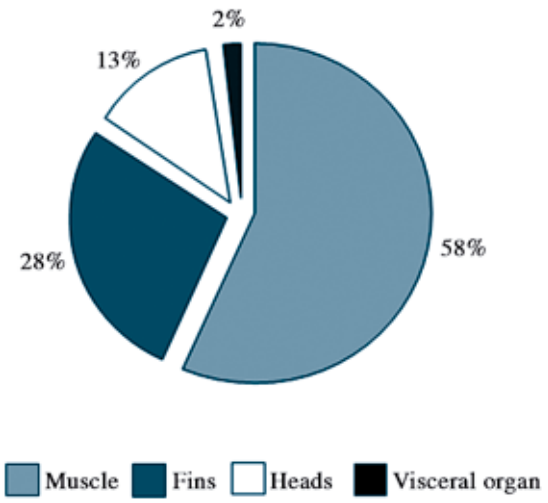

Fig 3 Distribution of *Opisthorchis viverrini* metacercariae in various parts of the fish body.

Table 3 Intensity of *O. viverrini* metacercariae (mtc) found in 8 species of cyprinoid fish from the 3 study sites.

Fish species	No. of fish infected	Total no. of mtc found	Mean ± SD number of mtc / g fish			
			Namdone	Nampakane	Mekong	Total
Cyclocheilichthys repasson	117	5,097	2.47 ± 1.08	2.83 ± 1.13	2.00 ± 0.0	2.63 ± 1.11
Cyclocheilichthys armatus	31	420	0.94 ± 0.47	0.94 ± 0.47	NA	1.05 ± 0.53
Cyclocheilichthys enoplos	6	125	1.25 ± 0.63	0.82 ± 0.32	NA	0.90 ± 0.43
Dangila lineata	68	3,732	2.78 ± 0.94	2.42 ± 1.01	NA	2.60 ± 0.98
Henicorhynchus lineatus	76	3,722	2.68 ± 1.05	2.26 ± 0.75	0.95 ± 0.63	2.4 ± 0.98
Hampala dispar	16	360	0.83 ± 0.65	1.01 ± 0.65	NA	0.92 ± 0.53
Puntioplites proctzysron	44	555	0.95 ± 0.45	0.92 ± 0.52	0.90 ± 0.44	0.93 ± 0.48
Osteochilus Waandersii	11	70	1.97 ± 0.45	2.20 ± 0.00	NA	1.90 ± 0.45
Total	369	14,081	2.33 ± 1.12	2.18 ± 1.16	1.03 ± 0.55	2.2 ± 1.15

various species of freshwater fish, including (Laotian name in brackets) *Hampala dispar* (Pa-sout), *Amblynchichthus struncatu* (Pa-mang), *A. strunca* (Pa-tapo), *Cyclocheilichthys armatus* (Pa-kawna), *C. enoplos* (Pa-chok), *Discherodontusahme* (Pa-hangdeng), *Garrafuliginosa fowler* (Pa-khome), *Hypsibarbus pierrei* (Pa-park), *Lobocheilus melanotaen* (Pa-langnam), *Oreinchthys patvus* (Pa-siew-na), *Osteochilus waadersii* (Pa-Chiw), *O. hasselti* (Pa-itai), *Parachela maculicauda* (Pa-tebhuabird), *Poropuntus laoensis* (Pa-chat), *Puntius brevis* (Pa-khaomon), *Puntioplites falcifer* (Pa-sakang), *Raiamas guyttatua* (Pa-mitsanak-noy), *Rasbora ourodontusahme* (Pa-siew-our), and *Systomus orphoides* (Pa-pok) [7,9]. In the present study, 3 of 8 species of freshwater fish were found positive for *O. viverrini* metacercariae–*D. lineate* (Pa-kulum), *H. lineatus* (Pa-lankong), and *O. waandersii* (Pa-chewkoun)–which were thus confirmed as new second intermediate hosts for *O. viverrini* in Khammoune Province, according to previous reports.

In the present study, the *O. viverrini* metacercarial infection rates in the fish studied were high, especially among 2 fish species, *C. repasson* and *D. lineate,* collected from Namdone and Nampakane rivers, which were heavily infected with *O. viverrini*, metacercariae, whereas infections among specimens from the Mekong were low. The mean intensities of *O. viverrini* metacercariae in the fish from the 3 rivers were high in 3 species of freshwater fish, *C. repasson, D. lineate,* and *H. lineatus*. *O. viverrini* were mainly distributed in the body muscles of the cyprinoid fish collected. The result of this study corresponded with Vichasri (1982) [11], who reported that most *O. viverrini* metacercariae (> 90%) were found in the body muscles of cyprinoid fish in Thailand. In this study, cyprinoid fish were found to be heavily infected during the dry season; this result also agrees with Vichasri [11], who reported that the metacercarial burden in fish in Thailand peaked during the dry season.

Observations indicate that many factors influence disease transmission in these areas, such as the existence of opisthorchiasis among Laotian people living along the Namdone, Nampakane, and Mekong rivers, poor sanitation, and latrine use, and the persistent habit of eating raw fish. Other factors also support disease transmission, such as low water levels in the rivers, slowly running water, the ease of catching fish, and the abundance of snail intermediate hosts in the rivers in the dry season, especially in the Namdone and Nampakane rivers. These findings revealed that Laotian people who prefer to eat raw fish, especially cyprinoid fish captured from the Namdone and Nampakane rivers in the dry season, are at high risk of *O. viverrini* infection. The result of this study was a practical understanding of fish-borne trematode infections in Khammouane Province, Lao PDR. High rates and intensities of *O. viverrini* metacercarial infection among certain species of cyprinoid fish were demonstrated in the Namdone, Nampakane, and Mekong rivers.

Further research studies in endemic areas of Khammouane Province, Lao PDR, should consider interventions using health-education and sanitation-improvement approaches in the liver-fluke control program, to change eating and defecation habits, and thereby reduce the occurrence of *O. viverrini* infection.

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References

1. Keiser J, Utzinger J. Emerging foodborne trematodiasis. Emerg Infect Dis. 2005;11: 1507-14.
2. WHO. Control of food borne trematode infection. WHO Tech Rep Ser. 1995;849:1-157.
3. WHO. Report of joint WHO/FAO workshop on food-borne trematode infections in Asia, Ha Noi, Vietnam; 2002 November 26-28. WHO, WPRO. 2004;1-58.
4. Bedier E, Chesneau P. Distomatose hepatique a Opisthorchis in Laos (Vientiane and Thakek). Bull Soc Path Exot. 1929;22:331-4.

5. Upatham ES, Viyanant V. *Opisthorchis viverrini* and opisthorchiasis: A historical review and future respective. Acta Trop. 2003;88:171-6.
6. Kobayashi J, Vannachone B, Sato Y, Manivong K, Nambanya S, Inthakone S. An epidemiological study on *Opisthorchis viverrini* infection in Lao villages. Southeast Asian J Trop Med Public Health. 2000;31:128-32.
7. Rim HJ, Chai JY, Min DY, Cho SY, Eom KS, Hong SJ, *et al*. Prevalence intestinal parasite infection on a national scale among primary schoolchildren in Laos. Parasitol Res. 2003;91:267-72.
8. Ditrich O, Scholz T, Giboda M. Occurrence of some medically important fluke (Trematoda: Opisthorchiidae and Heterophyidae) in Nam Ngum water reservoir, Laos. Southeast Asian J Trop Med Public Health. 1990;21:482-8.
9. Sayasone S, Odermatt P, Phoumindr N, Vongsaravane X, Sensombath V, Phetsouvanh R, *et al*. Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. Trans R Soc Trop Med Hyg. 2007;101:40-7.
10. Kaewkes S. Taxonomy and biology of liver flukes. Acta Trop. 2003;88:177-86.
11. Vichasri S, Viyanant V, Upatham ES. *Opisthorchis viverrini*: intensity and rates of infection in cyprinoid fish from endemic focus in Northeast Thailand. Southeast Asia J Trop Med Public Health. 1982;13:138-41.