

Prevalence of Helminthic Infections among Inhabitants of Lao PDR

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Abstract: The prevalence of helminthic infections was surveyed on a total of 6,178 residents (males 2,549 and females 3,629) in 102 villages of 9 provinces in Lao PDR between 2007 and 2011 under the project of Korea-Laos Collaborative Project for Control of Foodborne Trematode Infections in Lao PDR. Fecal specimens were collected and examined by the Kato-Katz thick smear and Stoll's egg counting techniques. The overall liver/intestinal helminth egg positive rate was 71.9% with a single or mixed infections with *Opisthorchis viverrini* and minute intestinal flukes (Ov/MIF), *Ascaris lumbricoides*, hookworms, *Trichuris trichiura*, *Trichostrongylus* sp., echinostomes, *Taenia* spp., and others. Ov/MIF revealed the highest prevalence (55.6%) followed by hookworms (27.8%) and *T. trichiura* (6.5%). The endemic regions with the highest prevalence of Ov/MIF were Savannakhet, Khammouane, Vientiane (Nam Ngum), Champasak (Khong Island), and Saravane Province. High prevalences of *A. lumbricoides* (33.8%), hookworms (47.8%), and *T. trichiura* (32.6%) were observed in Phongsaly, Luang Prabang, and Vientiane (Nam Ngum) areas, respectively. The results of this study highlight helminth parasites of current public health significance in different areas of Lao PDR.

Key words: *Opisthorchis viverrini*, foodborne trematode, intestinal helminth, liver fluke, minute intestinal fluke, Lao PDR

INTRODUCTION

Soil-transmitted helminths and foodborne trematodes are important human parasites in Lao People's Democratic Republic (Lao PDR) [1], and liver and intestinal flukes causing foodborne trematodiasis are endemic in Lao PDR, Cambodia, Thailand, and Vietnam [2]. Previous studies have reported high prevalence of intestinal parasite infections among government staff and schoolchildren in different localities of Lao PDR. For example, the prevalence of intestinal helminths was 77.3% among schoolchildren below 15 years of age in Khammouane Province [3], 75.9% and 63.8% among government staff and schoolchildren in Champasak Province [4], 56.7% among chil-

dren below 15 years of age in Bolikhamxay Province, and 81.9% in Khammouane Province [5,6]. In a nationwide survey of primary schoolchildren aged 6-11 conducted between 2000 and 2002, the overall prevalence of helminth infections was 61.9% [1]. The intestinal helminth eggs/larvae observed in previous studies included *Ascaris lumbricoides*, hookworms, *Trichuris trichiura*, *Strongyloides stercoralis*, *Opisthorchis viverrini*, *Taenia* sp., and *Hymenolepis* spp.

Studies from neighboring countries along the Mekong river basin, in particular Thailand, Cambodia, and Vietnam, have reported high prevalences of fish-borne parasites. In previous studies on Lao PDR, the prevalence of fish-borne helminth infections detected by stool examination was 67.1% and 81.1% in Savannakhet and Khammouane Province, respectively [7,8]. Worm recovery revealed mixed infections with various helminths, including hookworms, *T. trichiura*, *A. lumbricoides*, *Enterobius vermicularis*, *Trichostrongylus* spp., *Taenia saginata*, echinostomes, *O. viverrini*, *Haplorchis taichui*, *Haplorchis pumilio*, *Haplorchis yokogawai*, *Prosthodendrium molenkampi*, and *Pha-*

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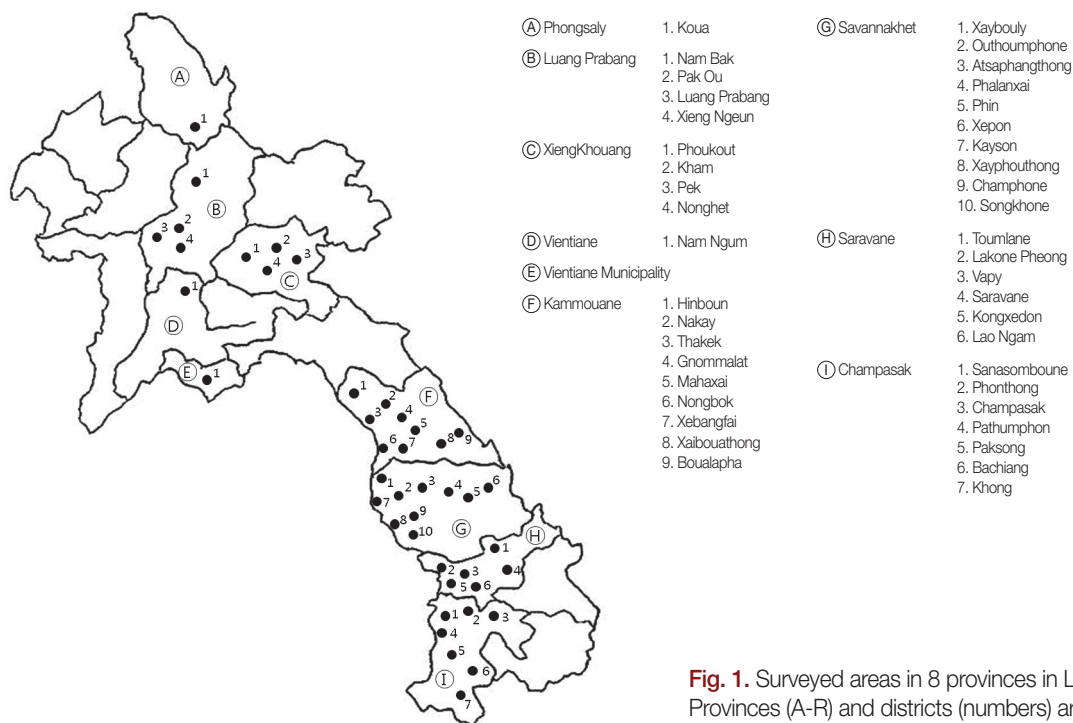


Fig. 1. Surveyed areas in 8 provinces in Lao PDR. Provinces (A-I) and districts (numbers) are shown.

neropsolus bonnei [7,8].

Helminthic infections are one of the neglected zoonotic diseases typically affecting rural communities in the developing world. Foodborne liver flukes, including *O. viverrini*, *Opisthorchis felineus*, and *Clonorchis sinensis*, cause morbidity and mortality in people and can cause a serious socioeconomic impact [2]. Due to the strong tradition of the Laotian people eating raw freshwater fish, exposure to liver flukes in Lao PDR is high, particularly for those residing in the Mekong river basin.

The aim of the present study was to undertake a parasitological survey to elucidate the diversity and prevalence of helminthic infections in Lao PDR and to identify species of current public health significance.

MATERIALS AND METHODS

Areas and populations surveyed

A total of 6,178 fecal specimens, 1 sample from each person, were collected from residents (males 2,549 and females 3,629) of 102 villages in 43 districts within 9 provinces of Lao PDR, between the years 2007 and 2011. Surveys were conducted in Vientiane Municipality (n=527) in December 2007, Savannakhet (n=1,109) Province in June 2008, Savannakhet (n=572) and Phongsaly (n=65) Province in December 2008, Khammouane Province (n=833) in June 2009, Saravane Province

(n=778) in December 2009, Champasak Province (n=669) in June 2010, Champasak (Khong Island) (n=282) and Vientiane/Nam Ngum Dam (n=144) in December 2010, Xieng Khouang (n=642) in June 2011, and Luang Prabang (n=557) in December 2011 (Fig. 1).

Fecal examinations

Fecal specimens were examined for intestinal helminth eggs using the Kato-Katz smear technique or Stoll's dilution egg counting method. Eggs of *O. viverrini* and minute intestinal flukes (MIF) that could not be differentiated only by morphology were recorded collectively as Ov/MIF.

RESULTS

The overall helminth egg positive rate was 71.8% (4,439/6,178) (Table 1). The most frequently encountered was *O. viverrini* and MIF (Ov/MIF), with an egg positive rate of 55.6%. Eggs of other parasites included *A. lumbricoides* (3.7%), hookworms (27.8%), *T. trichiura* (6.5%), *Trichostrongylus* sp. (4.6%), *Taenia* spp. (4.8%), and echinostomes (0.7%) (Table 1). Eggs of other helminths, e.g., *E. vermicularis* and *Schistosoma mekongi* were also observed in a few cases. Although the most frequently encountered eggs in the study were those of Ov/MIF (55.6%), 3 localities had higher prevalences of other intestinal helminths,

Table 1. Prevalence and intensity of intestinal helminth infections in 9 provinces of Lao PDR (2007-2011)

Year	Locality (districts/villages)	No. of exam. (male/female)	Prevalence of intestinal helminth infections (%)																							
			Ov/MIF (male/female)			Al (male/female)			Hw (male/female)			Tt (male/female)			Trichostrongylus sp. (male/female)			Ech (male/female)			Ts (male/female)					
			No. of positive (%) (male/female)	Light infection	Moderate infection	Heavy infection	No. of positive (%) (male/female)	Light infection	Moderate infection	Heavy infection	No. of positive (%) (male/female)	Light infection	Moderate infection	Heavy infection	No. of positive (%) (male/female)	Light infection	Moderate infection	Heavy infection	No. of positive (%) (male/female)	Light infection	Moderate infection	Heavy infection	No. of positive (%) (male/female)	Light infection	Moderate infection	Heavy infection
2007	Vientiane Municipality (1/8)	527 (201/326)	306 (65.1) (119/187)	241 (78.8) (93/148)	60 (19.6) (25/35)	5 (1.6) (1/4)	2 (0.4) (1/1)	1 (0.0) (0/1)	1 (0.0) (0/1)	1 (0.0) (0/1)	22 (6.8) (10/12)	8 (2.5) (4/4)	2 (0.6) (1/1)	8 (1.5) (3/5)	7 (87.5) (2/5)	1 (1.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	1 (0.2) (1/0)	1 (0.0) (0/0)	1 (0.0) (0/0)	1 (0.2) (1/0)	1 (0.0) (0/0)	1 (0.0) (0/0)	4 (0.9) (2/2)	24 (4.6) (7/17)
2008-1	Savannakhet (A) (6/18)	1,109 (423/686)	714 (64.4) (288/426)	470 (65.8) (187/283)	197 (27.6) (81/116)	47 (6.6) (20/27)	1 (0.1) (0/1)	1 (0.0) (0/0)	1 (0.0) (0/0)	1 (0.0) (0/0)	364 (32.8) (139/225)	259 (71.2) (43/57)	5 (1.4) (0/5)	11 (1.0) (5/6)	11 (100) (5/6)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	19 (1.7) (8/11)	1 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	19 (1.7) (10/9)	10 (0.9) (6/4)	75 (6.8) (30/45)	
2008-2	Savannakhet (B) (4/10)	572 (254/318)	482 (66.0) (224/258)	433 (75.7) (121/154)	136 (31.4) (69/68)	22 (5.1) (9/13)	2 (0.3) (2/0)	1 (0.0) (1/0)	1 (0.0) (1/0)	1 (0.0) (1/0)	229 (40.0) (88/131)	141 (61.6) (36/50)	2 (0.9) (0/2)	32 (6.5) (14/18)	32 (100) (14/18)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	71 (12.4) (31/40)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	71 (12.4) (31/40)	5 (0.9) (3/2)	30 (1.9) (0/0)	
	Phongsavay (1/1)	65 (19/46)	41 (63.1) (13/28)	5 (7.7) (3/2)	0 (0.0) (0/0)	0 (0.0) (0/0)	22 (33.8) (5/17)	2 (0.0) (0/0)	2 (0.0) (0/0)	24 (36.9) (7/14)	3 (1.2) (1/2)	0 (0.0) (0/0)	18 (27.7) (8/10)	18 (100) (8/10)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	
2009-1	Khammuane (9/16)	833 (398/435)	694 (83.3) (322/372)	411 (64.3) (203/208)	195 (30.5) (89/107)	33 (5.2) (21/12)	23 (2.8) (9/14)	5 (21.7) (3/2)	2 (8.7) (0/2)	166 (79.0) (79/87)	43 (20.5) (20/23)	1 (0.5) (0/1)	83 (10.0) (47/36)	82 (98.8) (47/35)	1 (1.2) (0/1)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	51 (6.1) (28/23)	17 (2.0) (10/7)	1 (0.0) (0/0)	0 (0.0) (0/0)	17 (2.0) (10/7)	59 (7.1) (41/18)		
2009-2	Saravane (6/12)	778 (286/492)	620 (79.7) (234/386)	451 (80.5) (165/286)	104 (18.6) (41/63)	5 (0.9) (5/0)	31 (4.0) (12/19)	1 (3.2) (0/1)	0 (0.0) (0/0)	182 (23.4) (62/120)	144 (79.1) (48/96)	2 (1.1) (0/2)	45 (6.3) (21/24)	43 (85.6) (1/1)	2 (4.4) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	20 (2.6) (10/10)	2 (0.3) (0/2)	0 (0.0) (0/0)	0 (0.0) (0/0)	20 (2.6) (10/10)	2 (0.3) (0/2)	35 (4.5) (12/23)	
2010-1	Champassak/Pakse (6/12)	669 (269/400)	439 (65.6) (188/251)	290 (89.0) (129/161)	34 (10.4) (15/19)	2 (0.6) (2/0)	40 (6.0) (11/29)	1 (2.5) (0/1)	0 (0.0) (0/0)	151 (22.6) (65/86)	114 (75.5) (51/63)	3 (2.0) (0/3)	17 (2.5) (6/11)	17 (100) (6/11)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	53 (7.9) (26/27)	1 (0.1) (0/1)	0 (0.0) (0/0)	0 (0.0) (0/0)	53 (7.9) (26/27)	1 (0.1) (0/1)	21 (3.1) (8/13)		
2010-2	Champassak/Khong (1/8)	282 (114/168)	251 (89.0) (101/150)	131 (54.6) (53/78)	88 (86.7) (35/53)	21 (8.8) (8/13)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	35 (12.4) (17/18)	29 (82.9) (3/2)	1 (2.9) (1/0)	4 (1.4) (1/3)	4 (100) (1/3)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	44 (15.6) (25/19)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	44 (15.6) (25/19)	0 (0.0) (0/0)	8 (2.8) (6/5)		
	Vientiane (Nam Ngum) (1/3)	144 (67/77)	110 (76.4) (50/60)	99 (68.8) (45/54)	20 (20.2) (9/11)	1 (1.0) (1/0)	28 (19.4) (12/16)	7 (25.0) (2/5)	0 (0.0) (0/0)	17 (11.8) (8/9)	15 (88.2) (7/8)	1 (5.9) (0/1)	47 (32.6) (25/22)	47 (100) (25/22)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	2 (1.4) (1/1)	2 (1.4) (1/1)	0 (0.0) (0/0)	0 (0.0) (0/0)	2 (1.4) (1/1)	1 (0.7) (0/1)	0 (0.0) (0/0)		
2011-1	Xiang Khouang (4/14)	642 (288/354)	285 (44.3) (127/158)	28 (4.4) (21/7)	26 (92.9) (19/7)	2 (7.1) (2/0)	36 (5.6) (13/23)	7 (19.4) (2/5)	0 (0.0) (0/0)	209 (32.6) (97/112)	135 (64.6) (24/31)	19 (9.1) (8/11)	47 (7.3) (26/21)	46 (97.9) (25/21)	1 (2.1) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	4 (0.6) (2/2)	10 (1.6) (8/2)	
2011-2	Luang Prabang (4/10)	557 (230/327)	363 (65.2) (150/213)	85 (15.3) (46/39)	84 (98.8) (46/38)	1 (1.2) (0/1)	42 (7.5) (12/30)	8 (19.0) (0/8)	0 (0.0) (0/0)	266 (47.8) (110/156)	173 (65.0) (74/99)	16 (6.0) (6/10)	92 (16.5) (39/53)	91 (98.9) (39/53)	1 (1.1) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	24 (4.3) (11/13)	0 (0.0) (0/0)	0 (0.0) (0/0)	0 (0.0) (0/0)	24 (4.3) (11/13)	0 (0.0) (0/0)	15 (2.7) (8/7)		
Total (43/102)		6,178 (2,549/3,629)	4,439 (71.9) (1,584/2,855)	3,435 (55.6) (1,495/1,940)	2,462 (71.7) (1,064/408)	136 (4.0) (37/4/63)	227 (3.7) (77/150)	33 (14.5) (9/24)	2 (0.8) (0/2)	1,719 (27.9) (718/1,001)	1,219 (70.9) (512/707)	448 (26.1) (188/259)	52 (3.0) (17/35)	404 (6.5) (186/209)	6 (1.5) (4/2)	0 (0.0) (0/0)	0 (0.0) (0/0)	285 (4.6) (141/144)	44 (0.7) (24/20)	0 (0.0) (0/0)	0 (0.0) (0/0)	285 (4.6) (141/144)	44 (0.7) (24/20)	296 (4.8) (147/149)		

Ov/MIF: Opisthorchis viverrini/minute intestinal flukes (mostly Hapocryptus tachui); Al: Ascaris lumbricoides; Hw: hookworms; Tt: Trichostrongylus trichiura; Ech: Echinostoma; Ts: Taenia spp.

DISCUSSION

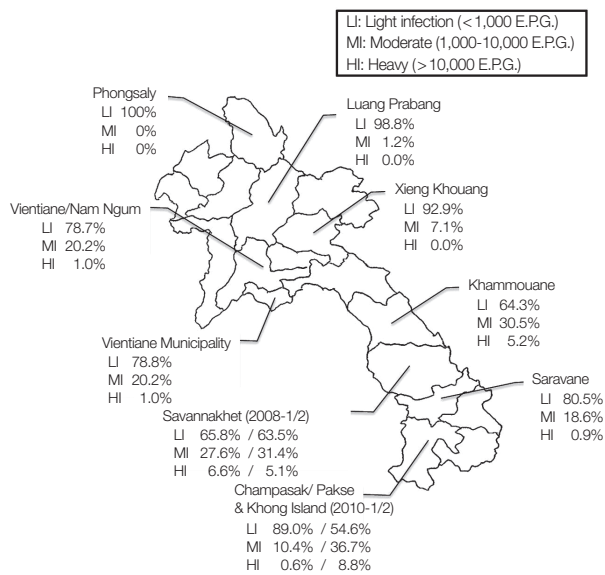


Fig. 2. Map showing the prevalence and intensity of *Opisthorchis viverrini* and minute intestinal flukes in Lao PDR.

e.g., *A. lumbricoides* (33.8%) in Phongsaly, hookworms (47.8%) in Luang Prabang, *T. trichiura* (32.6%) in Vientiane/Nam Ngum. *S. mekongi* eggs were found only in Champasak/Khong Island region (Table 1). The Ov/MIF endemic regions with the highest prevalence were those located in the Mekong river basin, e.g., Savannakhet (64.4-75.7%), Khammouane (76.7%), Vientiane/Nam Ngum (68.8%), Champasak/Pakse (48.7-85.1%) and Saravane Province (72.0%). Among them, Champasak/Pakse and Saravane Province turned out to be mixed endemic regions of MIF and *O. viverrini*.

Overall light (<1,000 epg), moderate (1,000-10,000), and heavy (>10,000 epg) infections of *O. viverrini* and MIF shared 71.7%, 24.4%, and 4.0%, respectively (Table 1). Heavy infections of Ov/MIF were noticed in several provinces such as Savannakhet (6.6%), Khammouane (5.2%), and Champasak/Khong (8.8%) (Fig. 2). High prevalences of soil-transmitted helminths were also recorded, although most cases were detected with light infections. Light (<5,000 epg), moderate (5,000-50,000 epg), and heavy (>50,000 epg) infections of *A. lumbricoides* were 84.1%, 14.5%, and 0.8%, respectively. Light (<400 epg), moderate (<3,000 epg) and heavy (>3,000 epg) infection of hookworms were 70.9%, 26.1%, and 3.0%, respectively. Light (<1,000 epg), moderate (1,000-10,000 epg) and heavy (>10,000 epg) infections of *T. trichiura* were 98.5%, 1.5%, and 0%, respectively (Table 1).

The overall prevalence (71.9%) of helminth infections in this survey was somewhat higher than that (61.9%) reported in a previous study in various localities of Lao PDR in 2000-2002 [1]. The remarkable difference in the 2 surveys was much higher prevalence of Ov/MIF eggs in this survey and much decreased prevalence of soil-transmitted helminths, in particular, *A. lumbricoides* and *T. trichiura*, in this survey [1].

In 2000-2002, the prevalence of *A. lumbricoides* infection was very high in Phongsaly (81.9%), Saysamboune (73.4%), and Huaphan (72.0%), and averaged 34.9% nationwide. However, in the present study, the prevalence of *A. lumbricoides* became much decreased, with the average prevalence becoming only 3.6%. The highest prevalence was found in Phongsaly (33.8%) and Vientiane (Nam Ngum) Province (19.3%). Though not comparable with *A. lumbricoides*, the prevalence of *T. trichiura* also showed a remarkable decrease. The average prevalence in 2000-2002 was 25.8% [1] but it was reduced to 6.5% in 2007-2011 (this study).

However, with regard to hookworm infections, the average prevalence in 2000-2002 was 19.1% but that in this study was 27.8%, a somewhat increased figure. In particular, the hookworm prevalence remained over 30% in 4 provinces including Luang Prabang, Xieng Khouang, Phongsaly, and Savannakhet Province. This represents no decrease in the hookworm prevalence in many localities of Lao PDR during the past 7-9 years.

The prevalence of Ov/MIF in 2000-2002 was 10.9% [1]. However, in this study, Ov/MIF infection was found to be 55.6%, almost 5-fold higher than the figure of 7-9 years before. This can be explained that the present survey was performed mainly in known endemic areas of foodborne helminths such as Ov/MIF, whereas the previous survey was performed just nationwide. It is of note that, by worm recovery studies, Saravane and Champasak Province was verified to be an endemic area of MIF (e.g., *Haplorchis taichui*) only with a low grade *O. viverrini* infection [9]. Agreeing to a previous study [7], the high prevalence (64.4-75.6%) of Ov/MIF in Savannakhet Province, was confirmed mainly due to *O. viverrini* infection by worm recovery from egg positive cases (to be published). The results of worm recovery in other provinces (Luang Prabang, Xieng Khouang, Khong Island, and Nam Ngum Dam areas) will also be reported separately.

Regarding *Taenia* tapeworm infections in Lao PDR, a total of 845 egg positive cases (including the present 296 egg positive

cases) and 126 adult *Taenia* specimens recovered from Laotian people in 17 provinces during 2000-2011 were analyzed [10]. The results revealed that the average prevalence of *Taenia* eggs was 1.5% (845/55,038) by the Kato-Katz technique [10]. Molecular analysis of the adult specimens using mitochondrial cytochrome *c* oxidase 1 (*cox1*) sequencing (440 bp) revealed that 3 of 126 specimens showed 99% similarity with *Taenia solium*, and the remaining 123 samples showed 99% similarity with *Taenia saginata* [10]. So far, no *Taenia asiatica* infections were found in Lao PDR.

In this study, most of the egg positive patients had mixed-infections with several kinds of intestinal helminth species, including heterophyids, lecitodendriids, and *O. viverrini*. The opisthorchiid liver flukes and heterophyid intestinal flukes are highly prevalent in endemic areas of southeast Asia, and *Taenia* spp. are also endemic in this region. Discrimination of these parasites in endemic regions based on egg morphology alone is difficult due to their morphological similarity. Over the past decade, the egg shell morphology has been used to distinguish the eggs of haplorchiid and opisthorchiid flukes [11]; however, it does not enable differentiation at a species level. More recently, PCR assays have been developed to improve the specificity and sensitivity of discriminating between *O. viverrini* and MIF species and cestodes also [12-14].

O. viverrini infection is known to be a major risk factor for cholangiocarcinoma. In Lao PDR, an estimated 2.5 million individuals are infected with *O. viverrini* [15]. A recent report suggested that *O. viverrini* infects 10 million people in Lao PDR and Thailand together [16]. The present study indicates that opisthorchiasis is highly endemic in Lao PDR, particularly in central and lowland areas, such as Vientiane, Khammouane, Savannakhet, and Khong Island. However, there were no well-organized control measures against opisthorchiasis in Lao PDR, and the incidence of cholangiocarcinoma in each endemic area has not been precisely known. To elucidate this and to establish strategies for prevention and control, determination of the relative predominance of *O. viverrini* and MIF in each specific endemic area is an important prerequisite.

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CONFLICT OF INTEREST

We declare no conflict of interest related with this work.

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