Risk Factors for *Opisthorchis viverrini* and Minute Intestinal Fluke Infections in Lao PDR, 2009–2011

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Abstract. The purpose of this report is to compare the prevalence of *Opisthorchis viverrini*/minute intestinal fluke (Ov/MIF) in Lao People's Democratic Republic according to different regions and evaluate the effect of ingestion of raw freshwater fish on the infection. Demographic data, behavioral data, and symptoms were obtained, and the Kato–Katz method was used for the diagnosis of Ov/MIF. In total, 3,046 individuals were enrolled. The risk factors associated with acquisition of Ov/MIF were raw fish consumption and province. Khong Island showed the highest prevalence (odds ratio = 80.1, 95% confidence interval = 47.9–133.9, $P < 0.001$) compared with other districts, and raw fish consumption was related to Ov/MIF infection. Administration of praziquantel to entire populations, education (avoiding consumption of raw food dishes and improved hygiene), and access to clean water and sanitation should be considered.

INTRODUCTION

The first human cases of *Opisthorchis viverrini* infection were reported in Thailand nearly 100 years ago.¹ *O. viverrini* is endemic in the Lower Mekong Basin, including Thailand, Lao People's Democratic Republic (Lao PDR), Cambodia, and central Vietnam.¹ Specifically, *O. viverrini* and other fluke infections (i.e., minute intestinal flukes [MIFs] and the blood fluke *Schistosoma mekongi*) and soil-transmitted helminths are endemic.²

The adult worm of *O. viverrini* inhabits the intra- and extra-hepatic biliary system. The embryonated eggs containing a miracidium released from the gravid worm are discharged with the bile and eventually passed into environment in the fecal stream. After reaching freshwater, the egg is ingested by snails of the genus *Bithynia*. The snails are abundant in water reservoirs with shallow water, rice fields, and wetlands. Within the snail, eggs hatch to release miracidia, which transform to sporocysts. Then, sporocysts undergo asexual reproduction to give rise to rediae and finally, cercariae. After the cercariae are released from snails, they attach to and penetrate the skin of freshwater fish (belonging to 1 of 20 susceptible species of cyprinids) from infective stage, the metacercaria. Humans are the major definitive host. People become infected when they ingest raw or inadequately cooked fish, such as koi pla (fish salad) and pla som (short fermented fish), particularly in Lao PDR. After ingestion of fish flesh, the encysted metacercariae are digested by gastric and intestinal juices, and excysted juvenile flukes migrate through the ampulla of Vater into the common bile duct and the intrahepatic bile duct, where they mature and sexually reproduce. Some flukes can be found in the common bile duct, cystic duct, and even the gallbladder.

In a recent survey, 70% of cyprinid fish caught from Lawa Lake, Khon Kaen Province, Thailand were found to be infected with *O. viverrini*. It has been assumed that cats and dogs act as reservoir hosts of *O. viverrini* in Thailand and Lao PDR.¹ Ten million people are infected with *O. viverrini* in Thailand and Lao PDR. The infections are associated with hepatobiliary diseases, including hepatomegaly, cholangitis, fibrosis of the periportal system, cholecystitis, and gallstones. They are the major etiological agents of bile duct cancer, cholangiocarcinoma (CCA), and as such, *O. viverrini* are classified as type 1 carcinogens by the International Agency for Research on Cancer, World Health Organization (IARC, WHO).¹

The habit of eating raw freshwater fish is supposed to be the risk factor of *O. viverrini* infestation, but study on this aspect is lacking. Therefore, the purpose of this report is to compare the prevalence of *O. viverrini* (Ov)/MIF in Lao PDR according to different regions and evaluate the effect of ingestion of raw freshwater fish on the infection.

MATERIALS AND METHODS

Study area. The study was carried out in five provinces along the Mekong River in Lao PDR between June of 2009 and December of 2011. Among these provinces, the selected villages of Xieng Khouang, Luang Prabang, and Champasak were far from the river, whereas Vientiane and Saravane were near the river. We surveyed Khong Island, the largest island of Si Phan Don (Four Thousand Islands), which is situated in a river stretch of the Mekong River, Khong District, Champasak Province, north of the border with Cambodia. The Mekong River is a transboundary river in southeast Asia, and the estimated length is 4,350 km.² From the Tibetan Plateau, this river runs through China's Yunnan province, Burma (Myanmar), Lao PDR, Thailand, Cambodia, and Vietnam (Figure 1). According to the 2005 census, the total population of Lao PDR was 5.62 million, of which 2.82 million were females and 2.80 million were males.³ The climate is of

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FIGURE 1. (A) Mekong River. (B) Prevalence of *O. viverrini*/MIF by area in Lao PDR from 2009 to 2011.

monsoon tropical type, with the rainy season occurring between May and October.

Subject and test. We investigated the population according to the villages and distributed the kits for stool samples in advance. A nurse in Lao PDR interviewed the patients on the day of stool sampling. Demographic (e.g., age, sex, and

education attainment), behavioral data (e.g., food consumption, smoking, and drinking), and symptoms were obtained from each participant using a questionnaire. Fecal specimens were examined for intestinal helminth eggs using the Kato-Katz smear technique that multiplies the number of eggs counted in the entire field of 41.7 mg pressed stool smear by 24 to obtain the number of eggs per gram. On the fecal examination result sheet, Ov actually means Ov/MIF, because the eggs of *O. viverrini* and MIFs could not be differentiated by morphology. Therefore, they were recorded as Ov/MIF in this paper.

Statistics. The data generated were coded, entered, validated, and analyzed using Statistical Package for Social Science (SPSS Inc., Chicago, IL), version 18.0. Odds ratios (ORs) in logistic regression with 95% confidence intervals (95% CIs) were calculated.

Ethical considerations. The study was approved by the Institutional Review Board of Eulji University Hospital in Daejeon, Korea. This study was approved by the Ministry of Public Health, Lao PDR under the agreement of the Korea-Lao PDR International Collaboration on Intestinal Parasite Control in Lao PDR (2002–2011). All participants provided written informed consent before study participation.

RESULTS

In total, 3,046 individuals were enrolled from Lao PDR. Table 1 summarizes the general characteristics of study subjects. There were more female than male participants (59.4% versus 40.6%). The order of the numbers of individuals was ages 40–49, 30–39, 50–59, 20–29, 60–69, 70+, and < 20 years, which was 777 (25.5%), 655 (21.5%), 653 (21.4%), 363 (11.9%), 351 (11.5%), 214 (7.0%), and 33 (1.1%), respectively. The participants were mostly above 20 years old. For the provinces, Saravane was highest (775; 25.4%) followed by Champasak (667; 21.9%), Xieng Khouang (640; 21.0%), Luang Prabang (540; 17.7%), Khong Island (280; 9.2%), and Vientiane (144; 4.7%) (Table 1).

The number of positive stools samples was 1331 (43.7%; male: 45.3% versus female: 42.6%). The positive stools were

TABLE 1
Distribution of study subjects by area, age, and sex in Lao PDR from 2009 to 2011

	Male		Female		Total
	<i>n</i>	Percent	<i>n</i>	Percent	
Province					
Xieng Khouang	286	44.7	354	55.3	640
Luang Prabang	221	40.9	319	59.1	540
Champasak	266	39.9	401	60.1	667
Vientiane	67	46.5	77	53.5	144
Saravane	283	36.5	492	63.5	775
Khong Island*	113	40.4	167	59.6	280
Total	1,236	40.6	1,810	59.4	3,046
Age, years					
< 20	19	57.6	14	42.4	33
20–29	113	31.1	250	68.9	363
30–39	255	38.9	400	61.1	655
40–49	313	40.3	464	59.7	777
50–59	284	43.5	369	56.5	653
60–69	146	41.6	205	58.4	351
70+	106	49.5	108	50.5	214
Total	1,236	40.6	1,810	59.4	3,046

*Khong Island is located in the Mekong River, Khong District, Champasak Province.

FIGURE 2. Prevalence of *O. viverrini*/MIF by area and raw fish consumption in Lao PDR from 2009 to 2011.

highest in Khong Island (239; 85.4%) followed by Saravane (558; 72.0%), Vientiane (99; 68.8%), Champasak (324; 48.6%), Luang Prabang (83; 15.4%), and Xieng Khouang (28; 4.4%). The highest age group was 30–39 years (316; 48.2%) followed by 40–49 (351; 45.2%), 60–69 (155; 44.2%), 70+ (93; 43.5%), 50–59 (272; 41.7%), 20–29 (133; 36.6%), and < 20 years (11; 33.3%). People with no education and primary education showed higher positive rates (344; 47.6% and 526; 50.5%) than people with secondary and college education (361; 38.8% and 95; 27.9%, $P < 0.001$). People who had jaundice-related symptoms or weight loss had low positive rates of Ov/MIF ($P < 0.05$); 31 of 46 people who had nausea/vomiting were positive for Ov/MIF (67.4%; $P = 0.001$). Among people who had consumed raw fish, 53.1% were positive for Ov/MIF. People who had no history of consumption of raw fish were 19.1% positive for Ov/MIF (Table 2). In multiple logistic regression analysis, the risk factors associated with acquisition of Ov/MIF were raw fish consumption and province. Khong Island showed highest prevalence (OR = 80.1, 95% CI = 47.9–133.9, $P < 0.001$) compared with other provinces, and raw fish consumption was significantly related to Ov/MIF infection (OR = 3.5, 95% CI = 2.7–4.5, $P < 0.001$) (Figure 2 and Tables 3).

DISCUSSION

Fish-borne trematode (FBT) infection is a public health problem in some Asian countries, like Lao PDR, where these flukes provoke remarkable morbidity. An estimated 2.5 million individuals are infected with *O. viverrini* in Lao PDR. These infections are highly localized depending on the food habits of residents and the presence of susceptible snail hosts.⁴ Lao PDR is located in the middle of southeast Asia and has the Mekong River flowing through the whole country from north to south. In Lao PDR, people residing in the Mekong River Basin, which occupies one-quarter of the territory, have some unique food habits, like consumption of raw fish, which can be infected with FBT. The MIFs are two families of trematodes: Heterophyidae (*Haplorchis taichui*, *H. pumilo*, and *Stellanchasmus falcatus*) and Lecithodendriidae (*Phaneropsolus bonnie* and *Prostodendrium molenkampii*).

TABLE 2

Prevalence of Ov/MIF by area and characteristics of subjects in Lao PDR from 2009 to 2011

	Total	Ov/MIF positive		Ov/MIF negative		P value
		n	Percent	n	Percent	
Province						< 0.001
Xieng Khouang	640	28	4.4	612	95.6	
Luang Prabang	540	83	15.4	457	84.6	
Champasak	667	324	48.6	343	51.4	
Vientiane	144	99	68.8	45	31.2	
Saravane	775	558	72.0	217	28.0	
Khong Island*	280	239	85.4	41	14.6	
Total	3,046	1,331	43.7	1,715	56.3	
Sex						0.139
Male	1,236	560	45.3	676	54.7	
Female	1,810	771	42.6	1,039	57.4	
Total	3,046	1,331	43.7	1,715	56.3	
Age, years						0.013
< 20	33	11	33.3	22	66.7	
20–29	363	133	36.6	230	63.4	
30–39	655	316	48.2	339	51.8	
40–49	777	351	45.2	426	54.8	
50–59	653	272	41.7	381	58.3	
60–69	351	155	44.2	196	55.8	
70+	214	93	43.5	121	56.5	
Total	3,046	1,331	43.7	1,715	56.3	
Education						< 0.001
No study	723	344	47.6	379	52.4	
Primary	1,042	526	50.5	516	49.5	
Secondary	930	361	38.8	569	61.2	
College	340	95	27.9	245	72.1	
Missing	11	5	45.5	6	54.5	
Total	3,046	1,331	43.7	1,715	56.3	
Symptoms						
Jaundice†	93	18	19.4	75	80.6	< 0.001
Weight loss	446	171	38.3	275	61.7	0.014
Nausea and vomiting	46	31	67.4	15	32.6	0.001
Raw fish						< 0.001
Yes	2,202	1,170	53.1	1,032	46.9	
No	844	161	19.1	683	80.9	
Total	3,046	1,331	43.7	1,715	56.3	
Smoking						< 0.001
Yes	1,015	546	53.8	469	46.2	
No	1,915	713	37.2	1,202	62.8	
Quit	89	53	59.6	36	40.4	
Missing	27	19	70.4	8	29.6	
Total	3,046	1,331	43.7	1,715	56.3	
Drinking						0.013
Yes	1,682	737	43.8	945	56.2	
No	1,021	425	41.6	596	58.4	
Quit	305	144	47.2	161	52.8	
Missing	38	25	65.8	13	34.2	
Total	3,046	1,331	43.7	1,715	56.3	

* Khong Island is located in the Mekong River, Khong District, Champasak Province.

† One or more symptoms among jaundice, dark urine, and yellow eyes.

MIF eggs and *O. viverrini* eggs show marked resemblance, and therefore, they are sometimes called *O. viverrini*-like eggs.⁴ To discriminate these eggs, molecular diagnosis, like polymerase chain reaction (PCR), is needed. In this study, Luang Prabang and Xieng Khouang showed low positive rates. The field survey showed that these areas are highly endemic with *Haplorchis* rather than *O. viverrini*.⁵ Therefore, the discrepancy between the positive rates would be higher in than this report.

In Thai populations, the fourth leading cause of mortality is liver and bile duct cancer,⁶ which shows the seriousness of these fluke infections. In northern Thailand, an estimated 5,000 cases of CCA are diagnosed annually, which can be prevented by education on eating habits.⁷ Most people with

TABLE 3

Risk of Ov/MIF infection using multiple logistic regression in Lao PDR from 2009 to 2011

	Total (n)	OR	95% CI		P value
			Lower	Upper	
Province					
Xieng Khouang	640	1.0			
Luang Prabang	540	3.2	2.0	5.1	< 0.001
Champasak	667	13.8	9.0	20.9	< 0.001
Vientiane	144	31.9	18.8	54.2	< 0.001
Saravane	775	53.9	35.4	82.0	< 0.001
Khong Island*	280	80.1	47.9	133.9	< 0.001
Sex					
Female	1,810	1.0			
Male	1,236	1.2	1.0	1.4	0.076
Raw fish consumption					
No	844	1.0			
Yes	2,202	3.5	2.7	4.5	< 0.001

Age, education, smoking, and drinking were not statistically significant, and they were excluded in the final model.

* Khong Island is located in the Mekong River, Khong District, Champasak Province.

opisthorchiasis have no symptoms. Only 5–10% of infected people (generally individuals with heavy fluke infections) have non-specific symptoms, such as right upper quadrant abdominal pain, flatulence, and fatigue.⁷ Our study showed that Ov/MIF-positive people had more nausea and vomiting. Enlargement of the gall bladder can be detected by ultrasonography, and it is reversed after elimination of flukes by praziquantel.⁷ Nonetheless, heavy long-standing infection is associated with a number of hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, fibrosis of the periportal system, cholecystitis, and cholelithiasis. Moreover, liver fluke infection is the etiology of the liver cancer subtypes CCA and cancer of the bile ducts.⁷ The incidence of CCA had a strong positive correlation with the prevalence of *O. viverrini* infection in Lao PDR.⁷ In 1994, the IARC of the WHO listed *O. viverrini* as a group 1 carcinogen; in other words, they considered it to be a direct risk factor for CCA.^{8,9} *O. viverrini*-induced liver cancer ranks first in mortality among cancers for men and second among cancers in women in the Mekong Basin subregion.^{1,10}

Three main mechanisms are proposed to contribute to CCA through chronic infection with *O. viverrini*: (1) mechanical damage to the biliary epithelia caused by the feeding activities of the parasites, (2) immunopathology caused by infection-related inflammation, and (3) toxic effects of parasite excretory/secretory molecules.⁷

The carbohydrate antigen CA-19-9 is widely used for diagnosis of CCA, but it lacks specificity, because it is also a marker for pancreatic cancer, gastric cancer, and primary biliary cirrhosis and is elevated in smokers.¹¹ Carcinoembryonic antigen, a marker for colorectal cancer, is elevated in approximately 30% of CCA patients.^{12,13} Some potential biomarkers for CCA are trypsinogen-2,¹⁴ mucin-5AC, and the soluble fragment of cytokeratin 19, although none are currently in clinical use.^{15,16}

The changes in *O. viverrini* associated with CCA include chronic inflammation and fibrosis combined with nitrate stress from either endogenous and/or exogenous nitrosamines, leading to DNA damage and fixation of mutations.⁷ Repeated infection of hamsters induced greater inflammation and more severe pathology in association with parasite-specific antibody during chronic inflammation, induced the expression of iNOS (inducible nitric oxide synthase) in inflammatory cells and the epithelium of bile ducts, and subsequently,

caused nitrosative and oxidative damage to nucleic acids. Repeated infection of hamsters followed by repeated chemotherapy with praziquantel resulted in increased inflammation but did not increase the induction potential of CCA.⁷ In naturally acquired human infections with *O. viverrini*, individuals who have been repeatedly infected and then repeatedly treated with praziquantel are exposed to continued antigenic assaults and presumably develop elevated parasite-specific antibody titers. When these individuals are exposed to nitrate stresses through endogenous and/or exogenous nitrosamines, they may be at increased risk of cholangiocarcinogenesis.⁷ In a case control study on the risk factors for *O. viverrini*-induced CCA and use of praziquantel, the OR of CCA increased significantly with repeated rounds of chemotherapy.⁷ This finding is particularly pertinent in areas such as Lao PDR, where mass drug administration is widespread and the incidence of CCA is higher than in Thailand, where chemotherapy is more intermittent. Only through a robust randomized controlled trial can such an important issue be resolved.

The identification of biomarkers of disease progression is also urgently required. If robust biomarkers that signal progression of chronic infection along the path of periductal fibrosis to CCA can be identified, targeted intervention strategies can be developed and rapidly deployed in endemic areas.⁷ Based on the findings describing the use of interleukin-6 (IL-6) as a marker for advanced periductal fibrosis and early CCA, the Thai Ministry of Public Health has introduced plasma IL-6 screening in conjunction with other tests to detect early liver cancer in *O. viverrini*-endemic areas of northeast Thailand.⁷ Fluke antigens, such as secreted proteases or granulin, have potential as intervention targets, including as vaccine candidates, given recent successes with chemotherapy targeting related enzymes in schistosomes.⁷

Our findings are in line with previous observations of high infection prevalence of *O. viverrini* in Saravane and Khammouane Provinces. The risk factors of Ov/MIF infection were province (especially Khong Island) and consumption of raw fish. Khong Island is surrounded by the Mekong River; therefore, the frequency of consumption of raw fish was high, and subsequently, the Ov/MIF-positive rate was significantly high. The reason for the 19.8% positive rate among people who had no history of consumption of raw fish was the contamination of food, hands, and surfaces and food preparation utensils with the infective stage of the parasite (i.e., metacercariae).^{17,18} Although the Ov/MIF-positive rate showed a higher trend in older people, people of all ages were at risk in Lao PDR. Second to tobacco use, infections are the most important preventable source of human malignancies.^{19–21} Administration of praziquantel to the entire populations should be considered as the first priority, particularly in the Mekong River corridor, to control morbidity. This intervention should be coupled with setting-specific information, education, and communication strategies (avoiding consumption of raw food dishes and improved hygiene) as well as access to clean water and sanitation.

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