RESEARCH ARTICLE

Prevalence and Factors Associated with *Opisthorchis viverrini* Infection in Khammouane Province, Lao PDR

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Abstract

***Opisthorchis viverrini* (OV) liver flukes are common parasites found in central and southern Laos and constitute a major public health problem in the country. Laos people continue to have the habit of extensively consuming raw or half-cooked fish which are intermediate hosts. This study aimed to determine the prevalence and factors associated with OV infection in the population of Thakek district, Khammouane Province. This cross-sectional analytic study covered 237 subjects who filled out structured questionnaires. Fecal examination for OV infection was performed by Kato's thick smear method. Data analysis was carried out using STATA Version 10.0. Multiple logistic regression was applied. The results showed that the infection rate of OV was 54.8 %. Factors associated with OV infections were gender, a habit of defecation in fields and raw fish (goi bplaa dip) consumption. Opisthorchiasis and associated cholangiocarcinoma development thus appear to remain as important concerns in Laos.**

Keywords: *Opisthorchis viverrini* - infection prevalence - influencing factors - Lao PDR

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Introduction

In many countries of South-East Asia infection with the liver fluke *Opisthorchis viverrini* (OV) is of considerable importance as a risk factor for cholangiocellular carcinoma (CC) development (Haswell-Elkins et al., 1994; Sriamporn et al., 2004; Sripa et al., 2011). In Viet Nam, *Clonorchis sinensis* may also be found (Doanh and Nawa, 2016). Infection with liver flukes (*Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis*) has been determined to be a class I carcinogen by the International Agency for Cancer Research evaluation committee (IARC, 1994). It has proved very difficult to change the dietary lifestyle habit of eating raw fish containing liver fluke eggs (Jongsuksuntigul and Imsomboon, 2003; Sripa et al., 2011) and a complicating factor is the presence of reservoir canine and feline hosts (Enes et al., 2010; Aunpromma et al., 2012). Furthermore, while the antihelminthic drug praziquantel may be used to control OV infection it maygive rise to an unwise dependency (Saengsawang et al., 2013) and repeated cycles of re-infection and treatment with praziquantel may increase the risk of CC (Kamsa-ard et al., 2015).

As in neighbouring Thailand and Cambodia (Sithithaworn et al., 2012), OV has long been known in Laos (Bedier and Chesnau, 1929) and more recent reports have confirmed its presence as a common parasite in central and southern parts of the country (Kobayashi et al., 2000; Sithithaworn et al., 2006; Sayasone et al., 2007; Forrer et al., 2012; Tomokawa et al., 2012). People in rural southern Laos still have the habit of consuming raw or half-cooked fish extensively (Xayaseng et al., 2013). The present study aimed to study the prevalence and factors associated with OV infection in the population in the Thakek district, Khammouane Province.

Materials and Methods

Design

The study design was a cross-sectional analytic study conducted in the Thakek district, Khammouane Province, Laos PDR, during the period June - July, 2015.

Subjects

There were 150 villages (88,229 population) in the Thakek district . 10 villages were randomized to be study samples. There were 3,494 population aged 15 year-old and above in these 10 villages. A required sample size of 237 was estimated using formulae for proportion estimation (Lemeshow et al., 1991) and multiple logistic regression (Hsieh et al., 1998). Systematic random sampling was applied to select the study subjects.

Data collection

All stool samples were analysed for the presence of OV eggs using the Kato's thick smear technique, and

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participants were interviewed by a research assistant using a structured questionnaire to obtain demographic information, habit of defecation, smoking, alcohol consumption and record the reported consumption (yes or no) of unsafely prepared fish, defined as one of five types of dishes containing raw freshwater fish and one containing freshwater fish which was likely to have been inadequately fermented. All participants found to be infected by OV were provided with a treatment dose of praziquantel.

Statistical analysis and ethical approval

Descriptive statistics were used to summarise the data. Associations between OV infection status and potential risk factors were analysed using unconditional logistic regression. Variables significant at the p<0.25 level on univariate analysis were included in multivariate analysis with backward elimination. Additional candidate variables were factors which were non-significant in the univariate analysis, but which have been reported as strong risk factors for OV infection in previous studies. Statistical analyses were performed using Stata version 10.0 (Stata Corp, 2007). Statistical significance in the final model was set at p≤0.05.

The research was approved by the Khon Kaen University Ethics Committee for Human Research (reference no. HE582126).

Results

Overall, the proportion of individuals positive for OV infection was 54.8%. Table 1 shows the results of univariate and multivariate analyses of factors contributing to risk of infection with Opisthorchis viverrini. Age, education level, past use of praziquantel and defecation practice were not found to be risk factors. While alcohol

Table 1. Factors Associated with Opisthorchis Viverrini (OV) Infection on Univariate and Multivariate Analysis

Factors	Number	%OV	Crude OR	95% CI	p-value	Adj. OR	95% CI	p-value
Overall	237	54.8						
Gender					<0.001			0.021
Female	112	36.6	1			1		
Male	125	71.2	4.28	2.48 - 7.39		2.61	1.16-5.89	
Age in years					0.150			0.873
≤35	75	48.0	1			1		
>35	162	58.0	1.50	0.86 - 2.60		1.18	0.33-2.10	
Education level					0.698			
Bachelor or Higher	31	51.6	1					
Primary, High school, none	206	55.3	1.16	0.54 - 2.47				
Occupation					0.084			0.109
Non-farmer	105	48.5	1			1		
Farmer, Labor	132	59.8	1.58	0.94 - 2.65		1.68	0.89-3.16	
Alcohol consumption					0.042			0.476
No	41.3	46.0	1			1		
Yes, current or previous	191	58.1	1.97	1.02 - 3.79		0.74	0.32-1.69	
Smoking					<0.001			0.108
No	145	41.4	1			1		
Yes	92	76.0	4.51	2.52 – 8.07		1.94	0.86-4.33	
Past use of praziquantel					0.281			0.188
Never taken	189	56.6	1			1		
Taken	48	47.9	0.70	0.37 - 1.33		0.59	0.27-1.29	
Defecate in toilets					0.060			0.085
All time	214	52.8	1			1		
Sometime, Never	23	73.9	2.53	0.96 - 6.67		2.70	0.87-8.35	
Defecate outside the home					0.194			0.412
Sanitary	57	47.4	1			1		
Insanitary	180	57.2	1.49	0.82 - 2.70		0.73	0.34-1.55	
Raw fish (gôi bplaa dip)					<0.001			<0.001*
No	61	21.3	1			1		
Yes	176	66.5	7.32	3.68 - 14.57		5.22	2.05 - 13.3	
Raw fish (lâap bplaa dip)					<0.001			0.714
No	56	32.1	1			1		
Yes	181	61.9	3.43	1.81 - 6.47		0.84	0.34-2.09	
Raw fish (sôm bplaa dip)					0.046			0.792
No	75	45.3	1			1		
Yes	162	59.3	1.75	1.01 - 3.05		0.89	0.39-2.04	
Raw fish (bplaa jòm dip)					0.001			0.245
No	108	43.5	1			1		
Yes	129	64.3	2.34	1.39 - 3.95		1.58	0.73-3.45	
Fermented fish (bplaa ráa dip)					0.173			0.621
No	22	40.9	1			1		
Yes	215	56.3	1.86	0.76 - 4.53		0.76	0.26-2.22	

*p-value<0.05, Goodness of fit=0.234

consumption and smoking were positive on univariate analysis this did not persist on multivariate analysis. Only gender and consumption of raw fish of type goi bplaa dip remained significant.

Discussion

The present study indicated that the infection rate of OV in our rural Laos community remains high and is directly related to gender and consumption of raw fish. The geographic pattern of OV infection is very uneven, but high rates are more likely in rural than urban environments, especially in wetlands and agricultural areas (Wattanayingcharoenchai et al., 2011). A common pattern that is emerging in each country of Mekong basin is the difference in transmission of OV between lowlands which have high prevalence versus highlands which have low prevalence (Sithithaworn et al., 2012).

In an earlier study in Laos, Champasack province, the rate was similar to that in the present study at 61.1% (Forrer et al., 2012). In Cambodia, while a low level of 4.6% was reported for Kratie Province (Sohn et al., 2012), in Takeo Province the percentage positive for OV was 46.4-50.6% (Yong et al., 2012). Four Cambodian provinces were identified as endemic areas of OV infection (Miyamoto et al., 2014). Reported rates in Thailand vary between 4.6% to 60.8% (Sithithaworn et al., 2012). High variability has also been reported between the different districts within provinces; for example, the age and gender adjusted proportion of the population infected in 20 districts of Khon Kaen Province varied between 10.0% and 70.9% (Sriamporn et al., 2004). Over the period 1981-2001 a variety of different surveys indicated that the Thai national prevalence of OV infection had fallen from 63.6% to 9.6%, but the most recent a data indicate the prevalence rate in the Northeast region remains high at 16.6% and had not decreased over the previous decade (Sithithaworn et al., 2012). In a review Kaewpitoon et al. (2015) reported 7 of 17 community-based surveys to have prevalences higher than 20%. and the highest was 45.7%. In upper Northeast Thailand, Nakhorn Phanom demonstrated a rate of 40.9% and four of seven provinces had a prevalence >20% (Thaewnongiew et al.,. 2014) .Rangsin et al. (2009) completed a two-year prospective survey of villagers of Chachoengsao Province in the Central region east of Bangkok and reported that the prevalence rate of OV infection increased from 21.3% to 26.2%.

However, it should be stressed that caution must be used in interpretation of data obtained by faecal egg examination. This method alone can lead to misidentification at the species level because of morphological similarity between the eggs of liver flukes and minute intestinal trematodes. In fact, recent surveys in Vietnam revealed that infection with several minute intestinal flukes, such as *Haplorchis pumilio* and *H. taichui*, are much more common than infection with *C. sinensis* or OV (Doanh and Nawa, 2016). Detection of OV-infection by urine OV-ES assay showed much greater diagnostic sensitivity and diagnostic specificity than the current "gold standard" FECT method for the detection and quantification of OV infection (Worasith et al., 2015) Immunodiagnosis of opisthorchiasis using parasite cathepsin F has been proposed (Teimoori et al., 2015) along with mitochondrial loop-mediated isothermal amplification (Le et al., 2012). PCR methods (tested in an experimental model (Wongratanacheewin et al., 2001), have been based on cytochrome c oxidase subunit one (cox1) and NADH dehydrogenase subunit one gene (nad1) (Buathong et al., 2015). Specific diagnosis of OV is also possible using loop-mediated isothermal amplification (LAMP) targeting copro-DNA (Arimatsu et al., 2012; 2015). One other approach is to apply a faecal parasite concentrator (Kaewpitoon et al., 2016). Another is identification of plasma protein tyrosine phosphatase alpha and fibronectin associated with liver fluke (Khoontawa et al., 2012). Earlier, repeated stool sampling and use of multiple techniques was found to enhance the sensitivity of helminth diagnosis in Laos (Savasone et al., 2015). Also in Laos, use of a handheld micoscope has been advocated (Bogoch et al., 2016).

Regarding risk factors for infection, in Champasack province of Laos age, Lao Loum ethnicity, educational attainment, occupation (i.e., rice farmer, fisherman, and animal breeder), and unsafe drinking water source have been identified as risk factors for infection (Forrer et al., 2012). A history of praziquantel treatment, access to sanitation, and distance to freshwater bodies were found to be protective factors (Forrer et al., 2012). Infection was significantly associated with the children's experiences of eating raw fish, frequency of eating raw fish by their guardians and maternal educational and career in southern Laos (Tomokawa et al., 2013). In Laos there is a low degree of knowledge among local people on the health risks related to frequent consumption of raw or insufficiently cooked fish (Xayaseng et al., 2013).In Thailand a history of OV infection, a history of taking praziquantel, and unsafe disposal of waste food were predictive of infection in one study (Chudthaisong et al., 2015). In another in North-East Thailand, male gender was found to be significantly and positively associated with OV infection while education to secondary school level or above was a significant protective factor, whereas eating of unsafely prepared fish and knowledge about OV were not significantly related to infection status (Chaiputcha et al., 2015). Age and gender were also singled out as importnat (Saengsawang et al., 2012) while smoking and alcohol consumption were associated with an increased chance of acquiring OV infection (Yeoh et al., 2015). Thaewnongiew et al. (2014) reported factors related to opisthorchiasis to be (a) sex, (b) age (especially > 50), (c) proximity and duration living near a water body, and (d) eating raw and/or fermented fish. A significant positive association with OV prevalence was found for the combined land use classes of water and of farmland with high water content, sugesting the need to incorporate land use planning into control strategies (Wang et al., 2013). Clearly avoidance of the metacercariae in cyrpinoid fish in Khammouane province (Khemphavanh et al., 2009) is a high priority. How this is to be achieved will undoubtedly be a focus of continuing research. Whether the local population can be persuaded to adopt pla-som fermentation for more than four days and refrigerating fish for three days before pla-som processing

which can prevent *O. viverrini* infection (Onsurathum et al., 2016) is one alternative which requires exploration, along with other community based-interventions.

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References

Arimatsu Y, Kaewkes S, Laha T, Hong SJ, Sripa B (2012). Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). *Parasitol Int*, **61**, 178-82

Arimatsu Y, Kaewkes S, Laha T, Sripa B (2015). Specific diagnosis of *Opisthorchis viverrini* using loop-mediated isothermal amplification (LAMP) targeting parasite microsatellites. *Acta Trop*, **141**, 368-71.

Aunpromma S, Tangkawattana P, Papirom P, et al (2012). High prevalence of *Opisthorchis viverrini* infection in reservoir hosts in four districts of Khon Kaen Province, an opisthorchiasis endemic area of Thailand. *Parasitol Int,* **61**, 60-4.

Bedier E, Chesneau P (1929). Distomatose hepatique a Opisthorchis in Laos (Vientiane and Thakek). *Bull Soc Path Exot*, **22**, 331-4.

Bogoch II, Sayasone S, Vonghachack Y, et al (2016). Diagnosis of *Opisthorchis viverrini* infection with handheld microscopy in Lao People's Democratic Republic. *Am J Trop Med Hyg*, **94**, 158-60.

Buathong S, Leelayoova S, Mungthin M, et al (2015). Development and evaluation of PCR methods based on cytochrome c oxidase subunit one (COX1) and NADH dehydrogenase subunit one gene (nad1) to detect *Opisthorchis viverrini* in human fecal samples. *Parasitol Res*, **114**, 3547-9.

Chaiputcha K, Promthet S, Bradshaw P (2015). Prevalence and risk factors for infection by *Opisthorchis viverrini* in an urban area of Mahasarakham province, northeast Thailand. *Asian Pac J Cancer Prev*, **16**, 4173-6.

Chudthaisong N, Promthet S, Bradshaw P (2015). Risk factors for *Opisthorchis viverrini* infection in Nong Khai province, Thailand. *Asian Pac J Cancer Prev*, **16**, 4593-6.

Doanh PN, Nawa Y (2016). *Clonorchis sinensis* and *Opisthorchis* spp. in Vietnam: current status and prospects. *Trans R Soc Trop Med Hyg*, **110**, 13-20.

Enes JE, Wages AJ, Malone JB, Tesana S (2010). Prevalence of *Opisthorchis viverrini* infection in the canine and feline hosts in three villages, Khon Kaen Province, northeastern Thailand. *Southeast Asian J Trop Med Publ Health*, **41**, 36-42.

Forrer A, Sayasone S, Vounatsu P, et al (2012). Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in Southern Lao PDR. *PLoS Negl Trop Dis*, **6**, 1481.

Haswell-Elkins MR, Mairiang P, Chaiyakhum J, et al (1994). Cross-sectional study of *Opisthorchis viverrini* infection and cholangiocarcinoma in communities within a high-risk area in northeast Thailand. *Int J Cancer*, **59**, 505-9.

Hsieh FY, Bloch DA, Larsen MD (1998). A simple method of sample size calculation for linear and logistic regresstion. *Statistics Med*, 17, 1623-34.

IARC (1994). Infection with liver flukes (*Opisthorchis viverrini*, Opisthorchis felineus and Clonorchis sinensis). *IARC Monogr Eval Carcinog Risks Hum*, **61**, 121-75.

Jamornthayawat N (2002). The diagnosis of human opisthorchiasis. *Southeast Asian J Tropical Med Public Health*; **33**, 86-91.

Jongsuksuntigul P, Imsomboon T (1997). The impact of a decade long opisthorchis control program in northeastern, Thailand. *Southeast Asian J Trop Med Public Health*, **28**, 551-7.

Kaewpitoon N, Kootanavanichpong N, Kompor P, et al (2015). Review and current status of *Opisthorchis viverrini* infection at the community level in Thailand. *Asian Pac J Cancer Prev*, **16**, 7375-9.

Kaewpitoon SJ, Rujirakul R, Tongtawee T, et al (2016). Detection of the carcinogenic liver fluke *Opisthorchis viverrini* using a mini parasep SF faecal parasite concentrator. *Asian Pac J Cancer Prev*, **17**, 373-376.

Kamsa-Ard S, Luvira V, Pugkhem A3, et al (2015). Association between praziquantel treatment and cholangiocarcinoma: a hospital-based matched case-control study. *BMC Cancer*, **15**, 776.

Khemphavanh M, Chalit K, Jitra W, Prayong R (2009). *Opisthorchis viverrini* metacercariae in cyprinoid fish from three river in Khammouane province, Lao PDR. *J Trop Med Parasitol*, **32**, 23-9.

Khoontawad J, Laothong U, Roytrakul S, et al (2012). Proteomic identification of plasma protein tyrosine phosphatase alpha and fibronectin associated with liver fluke, *Opisthorchis viverrini*, infection. *PLoS One*, 7:e45460.

Kobayashi J, Vannachone B, Sato Y, et al (2000). An epidemiological study on *Opisthorchis viverrini* infection in Lao villages. *Southeast Asian J Trop Med Public Health*, **31**, 128-32.

Le TH, Nguyen NT, Truong NH, De NV (2012). Development of mitochondrial loop-mediated isothermal amplification for detection of the small liver fluke *Opisthorchis viverrini* (Opisthorchiidae; Trematoda; Platyhelminthes). J *Clin Microbiol*, **50**, 1178-84.

Lemsehow S, HosmerJr WD, Klar JK, Lwanga S (1990). Adequacy of Sample Size in Health Studies. England: John Wiley & Sons;

Miyamoto K, Kirinoki M, Matsuda H, et al (2014). Field survey focused on *Opisthorchis viverrini* infection in five provinces of Cambodia. *Parasitol Int*, **63**, 366-73.

Onsurathum S, Pinlaor P, Haonon O, et al (2016). Effects of fermentation time and low temperature during the production process of Thai pickled fish (pla-som) on the viability and infectivity of *Opisthorchis viverrini Int J Food Microbiol*, **218**, 1-5.

Rangsin R, Mungthin M, Taamasri P, et al (2009). Incidence and risk factors of *Opisthorchis viverrini* infections rural community in Thailand. *Am J Trop Med Hyg*, **81**, 152-5.

Rhongbutsri P, Kitvatanachai S (2002). Survey of the fluke infection rate in Ban Khok Yai village, Khon Kaen, Thailand. *J Trop Med Parasitol*, **25**, 76-8.

Saengsawang P, Promthet S, Bradshaw P (2012). Prevalence of OV infection in Yasothon province, Northeast Thailand . *Asian Pac J Cancer Prev*, **13**, 3399-402.

Saengsawang P, Promthet S, Bradshaw P (2013). Infection with *Opisthorchis viverrini* use of praziquatel among a working-age population in Northeast Thailand. *Asian Pacific J Cancer Prev,* **14**, 2963-6.

Sayasone S, Odermatt P, Phoumindr N, et al (2007). Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. *Trans R Soc Trop Med Hyg*, **101**, 40-7.

Sayasone S, Utzinger J, Akkhavong K, Odermatt P (2015). Repeated stool sampling and use of multiple techniques enhance the sensitivity of helminth diagnosis: a cross-sectional survey in southern Lao People's Democratic Republic. *Acta Trop*, **141**, 315-21.

Sithithaworn P, Sukavat K, Vanachone B, et al (2006). Epidemiology of food-borne trematodes and other parasite infection in a fishing community on the Nam Ngum reservoir, Lao PDR. *Southeast Asian J Trop Med Public Health*, **37**, 1083-90.

Sithithaworn P, Andrews RH, Nguyen VD, et al (2012). The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitol Int*, **61**, 10-6.

Sohn WM, Yong TS, Eom KS, et al (2012). Prevalence of Opisthorchis viverrini infection in humans and fish in Kratie Province, Cambodia. *Acta Trop*, **124**, 215-20.

Sriamporn S, Pisani P, Pipitgool V, et al (2004). Prevalance of *Opisthorchis viverrini* infection and incidence of cholangiocarcinoma in KhonKaen, Northeast Thailand. *Tropical Med Int Health*, **9**, 588-94.

Sripa B, Bethony JM, Sithithaworn P, et al (2011). Opisthorchiasis and *Opisthorchis*-associated cholangiocarcinoma in Thailand and Laos. *Acta Trop*, **120**, 158-68.

Teimoori S, Arimatsu Y, Laha T, et al (2015). Immunodiagnosis of opisthorchiasis using parasite cathepsin F. *Parasitol Res*, **114**, 4571-8.

Thaewnongiew K, Singthong S, Kutchamart S, et al (2014). Prevalence and risk factors for *Opisthorchis viverrini* infections in upper Northeast Thailand. *Asian Pac J Cancer Prev*, **15**, 6609-12.

Tomokawa S, Kobayashi T, Pongvongsa B, et al (2012). Risk factors for *Opisthorchis viverrini* infection among schoolchildren in Lao PDR. *Southeast Asian J Trop Med Public Health*, **43**, 574-85.

Wang YC, Feng CC, Sithithaworn P (2013). Environmental determinants of Opisthorchis viverrini prevalence in northeast Thailand. *Geospat Health*, **8**, 111-23.

Wongratanacheewin S, Pumidonming W, Sermswan R, Maleewong W (2001). Development of a PCR-base method for the detection of *Opisthorchis viverrini* in experimentally infected hamsters. *Parasitol,* **122**, 175-180.

Worasith C, Kamamia C, Yakovleva A, et al (2015). Advances in the diagnosis of human opisthorchiasis: Development of *Opisthorchis viverrini* antigen detection in urine. **PLoS Negl Trop Dis**, **9**, e0004157.

Xayaseng V, Phongluxa K, van Eeuwijk P, Akkhavong K, Odermatt P (2013). Raw fish consumption in liver fluke endemic areas in rural southern Laos. *Acta Trop*, **127**, 105-11.

Yeoh KW, Promthet S, Sithithaworn P, Kamsa-Ard S, Parkin DM (2015). Re-examination of *Opisthorchis viverrini* infection in Northeast Thailand. *Asian Pac J Cancer Prev*, **16**, 3413-8.

Yong T-S, Shin E-H, Chai J-Y, et al (2012). High prevalence of *Opisthorchis viverrini* infection in a Riparian population in Takeo Province, Cambodia. *Korean J Parasitol*, **50**, 173-6.