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Prevalence and associated risk factors of *Opisthorchis viverrini* infections in rural communities along the Nam Kam River of Northeastern Thailand

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Abstract. *Opisthorchis viverrini* infection is still one of the public health problems in Southeast Asia. In Thailand alone, more than three million people are estimated to be currently infected. Opisthorchiasis may cause severe biliary diseases, eventually leading to fatal cholangiocarcinoma. This study aimed to investigate the prevalence and risk factors of opisthorchiasis in two rural communities along the Nam Kam River in the northeastern region of Thailand. A cross-sectional study was conducted in Phon Na Kaeo District of Sakon Nakhon Province and That Phanom District of Nakhon Phanom Province in Thailand between March and June 2017. Faecal samples were collected and examined for the presence of *O. viverrini* by Kato-Katz technique. Socio-demographic variables and risk factors were collected by a standardised questionnaire. The overall prevalence *of O. viverrini* among 495 study participants was 15.4% (95% confidence interval [CI] 12.3 – 18.8). The parasite rate was higher in Nakhon Phanom than in Sakon Nakhon (P = 0.009) and differed significantly in adults <45 years old in both provinces (P <0.05). Multivariate regression analyses revealed being labourer (adjusted odd ratio [AOR] 4.7 [95% CI 1.08, 20.38], P = 0.039) and farmer (AOR 5.79 [95% CI 1.45, 31.91], P = 0.015) were positively associated with a higher risk of *O. viverrini* infection. This study highlights *O. viverrini* infection as a significant endemic disease with potential health problems among the water-based communities along the Nam Kam River. For country with high *O. viverrini* transmission, such information will be useful for designing efficient strategic interventions.

INTRODUCTION

Opisthorchis viverrini, a fish-borne trematode, is one of the neglected tropical diseases with considerable public health importance in Southeast Asia, particularly in Thailand (Keiser & Utzinger, 2009; Sripa *et al*., 2010; Utzinger *et al*., 2010). It has been estimated that more than 3.3 million people in Thailand are infected with *O. viverrini* with varying distribution depending on the endemic areas (Wongsaroj *et al*., 2014). Rural communities in north and northeastern Thailand are the most effected with *O. viverrini* infections, where infection prevalence exceeding 50% at the village level is still commonly observed (Sithithaworn *et al*., 2012; Sripa *et al*., 2015). The concentrated distribution of *O. viverrini* in these regions corresponds with the local presence of natural intermediate hosts and the traditional eating habits (Sriamporn *et al*., 2004; Sripa *et al*., 2007). Apart from the northern areas, *O. viverrini* infection is also endemic in central Thailand due to the migration of local people from the northeastern provinces which

maintained their traditional life style that also includes raw food consumption (Rangsin *et al*., 2009; Suwannahitatorn *et al*., 2013).

The life cycle of *O. viverrini* is complex, involving two intermediate hosts: snails of the genus *Bithynia* are the first intermediate host, whereas freshwater fishes i.e. Cyprinoid family serve as the second intermediate host (Keiser & Utzinger, 2009). Human is infected by *O. viverrini* through consumption of raw or insufficiently cooked freshwater fish that harbours the infective stage of the parasites called metacercariae. Consumption of undercooked fish dishes such as *Koi pla* is deeply rooted in local culture in the affected areas, where proper sanitation is minimal or absent (Utzinger *et al*., 2010). Transmission is maintained by the frequent practice of open defecation that results in contamination of water bodies. Although infection with *O. viverrini* is often asymptomatic, chronic infection may lead to a number of severe liver and bile duct diseases (Mairiang & Mairiang, 2003; Keiser & Utzinger, 2009; Sripa *et al*., 2010). The most severe sequelae of *O. viverrini* infection is cholangiocarcinoma (CCA), a bile duct cancer with extremely poor prognosis (Sripa *et al*., 2010; Sripa *et al*., 2011) and with high fatality rate (Sithithaworn *et al*., 2014). In Thailand, previous studies have reported a high incidence rate of CCA in the northeastern regions ranging from 93.8 to 317.6 per 100,000 person-years (Sripa *et al*., 2007).

Today, preventive chemotherapy combined with health education via vertical programmes remain the main strategy for *O. viverrini* control in Thailand. It is implemented locally in target areas depending on problems and priorities in each community (Jongsuksuntigul & Imsomboon, 2003). Although most target areas are located in the northern and northeast regions which have been recognized as endemic area of *O. viverrini* infection, many reports showed that the prevalence was still high (Andrews *et al*., 2008; Ong *et al*., 2016; Kim *et al*., 2017; Saenna *et al*., 2017). In addition, despite decades of control efforts, the high availability of freshwater fish as major source of protein (Grundy-Warr *et al*., 2012), and the continuous practice of raw fish consumption among communities has led to the persistence of *O. viverrini* infections in many parts of these regions (Sripa *et al*., 2011; Sithithaworn *et al*., 2014).

This study was conducted in two districts along the Nam Kam River namely Phon Na Kaeo and That Phanom, in Sakon Nakhon Province and Nakhon Phanom Province respectively. Both provinces are situated in the northeastern region of Thailand approximately 500 km from the Thai capital Bangkok. Phon Na Kaeo District (352 km^2) is located at the downstream of the Nam Kam River, while That Phanom District (368 km^2) is about 80 km upstream of Phon Na Kaeo. The Nam Kam River is one of the Mekong River's tributaries which cover 3,440 km^2 of catchment area with a total length of 123 km. The river flows through two provinces starting from Nhong Harn swamp in Muang District of Sakon Nakhon and merges with the great Mekong River in That Phanom District in Nakhon Phanom. This river provides the water source for agriculture and for various utilities in this region. Though there are many developmental initiatives in this region, poverty is still a major challenge. Farming and fishing are the predominant economic activities for the river shore communities. Farming is mostly subsistence-based, particularly rice cultivation as well as small-scale animal husbandry. Fishing is done primarily by men in a variety of freshwater bodies including rivers, swamps and paddy fields. Dietary habits are deeply rooted in the local culture and include a variety of meals and dishes putatively containing metacercariae.

Previous epidemiological studies conducted in seven Upper Northeastern provinces in Thailand showed that the prevalence of *O. viverrini* varied among provinces: highest in Nakhon Phanom (40.9%), followed by Sakon Nakhon (~28%) and lowest in Bueng Kan (~10%) (Thaewnongiew *et al*., 2014). Nevertheless, according to the Province Statistic and Demographic Office, the total prevalence of *Opisthorchis* infections in Nakhon Phanom in 2016 and Sakon Nakhon in 2015 were lower than previously reported with 10.1% and 11.2%, respectively. These contrasting figures

implied that either intensive control measures of *Opisthorchis* infections by the health authority might have significant impact in the reduction of the infections or the overestimation of the true burden of *O. viverrini* in the study area. Furthermore, there is a dearth of information on the risk factors underlying each study area. The critical risk factors for *O. viverrini* infection are mainly associated with eating raw fish or uncooked fish. Differences in education, economy, social practices and life style amongst community has been found to influence the habit of consuming inadequately cooked fish (Sripa *et al.*, 2017).

In response, we aimed to investigate the current prevalence and associated risk factors of *O. viverrrini* infections using faecal samples from people living in Phon Na Kaeo and That Phanom, in Sakon Nakhon Province and Nakhon Phanom Province, respectively. Results from this study provide valuable information to direct improved health intervention efforts in order to identify and implement effective control measures, and plan for the participation of the targeted communities in the area.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Ministry of Health of Thailand (reference no. 5/2560) and the ethics committee of the Sawang Dan Din Crown Prince Hospital (reference no. SWDCPH 2017 – 009), which also approved the informed consent procedure. Permission for the field work was obtained from the Provincial Health Office and the District Health Office. Local health workers were briefed, using Thai language, on the participant information sheet and the need to obtain written consent from the participants, and on how to administer the questionnaire as well as to obtain faecal samples. Village meetings were held in the community hall centers and inhabitants were given detailed explanations about the aims, procedures, potential risks, and benefits of the study. Individual written informed consent was obtained from all adult household members. A witness observing this procedure also signed the consent form. For illiterate participant, informed consent was read out and, after approval, the person signed with a fingerprint. No children were involved in this study.

Study design and field procedure

A community-based survey was carried out between March and June, 2017. Two villages namely Ban Phon Khae Noi in Sakon Nakhon Province (Na Tong Watthana sub-district, Phon Na Kaew District) and Ban Fang Daeng in Nakhon Phanom Province (Fang Daeng sub-district, That Phanom District), were included in the study. These villages were selected based on, (i) their representativeness of typical lowland villages characterised by the proximity of water-bodies from which freshwater fish can be caught; (ii) maintenance of the tradition of eating raw fish dishes, which is typical for rural areas in the northeastern region of Thailand. The sample sizes for the different study sites were calculated using the following formula: $N = z^2 p (1 – p)/e^2$, where z is the confidence interval which is set at 95% (z-value of 1.96); p is the expected prevalence of *O. viverrini* infections of 11.2% for Sakon Nakhon and 10.1% for Nakhon Phanom (from available regional statistics) and e is the allowed error margin which is set to 4%. In addition, contingencies were adjusted for by adding another 10% of individuals, giving us a total of 503 participants to be faecal sampled for the two study sites combined.

Village leaders and household heads were informed about the study's objectives and procedures. A convenience sampling strategy was used in this study, whereby residents were asked to come to selected survey point for study participation. Enrolled participants were interviewed using standardised questionnaires to identify the risk factors for *O. viverrini* infection. The questionnaires covered socio-demographic aspects (i.e. age, gender, education level, marital status, occupation, income level, alcohol consumption as well as present and type of domestic animal) and behavioral aspects (i.e. raw fish consumption, previous

stool examination and positivity for helminth infection, praziquantel intake and prior knowledge about *O. viverrini* infection). With regards to the type of domestic animal, cats and dogs are the main animal reservoirs in the life cycle of *O. viverrini*, which they are allowed to roam freely in the study areas. All completed questionnaires were checked for accuracy and completeness.

After proper instruction, a biohazard zipper bag containing stool container and spatula, labeled with the subject's name and identification number was distributed to each participant. The participants were visited by local health workers at home the following day for collection of the samples. All collected samples were checked for correct labelling and quantity of sample, and transported immediately in a cool box to the field laboratory.

Parasitological data

Study participants were invited to provide a single stool sample. Modified Kato-Katz thick smears (Katz *et al*., 1972) were prepared after the stool collection using a commercially available kit (Department of Helminthology, Mahidol University, Bangkok, Thailand). The Kato-Katz technique is commonly employed in epidemiological surveys of helminth due to the relative ease of operation and the possibility to quantify infection intensity, which allows stratification into different intensity classes based on cut-offs provided by the World Health Organization (WHO) (2002). In this study, Kato-Katz thick smears were allowed to clear for 30 minutes before examination under a light microscope (100x magnification). To eliminate bias, each faecal sample was examined by three trained senior medical laboratory technologists who were not informed about the health status and other details of the study participants. All samples were examined on the day of collection. The number of eggs per gram faeces (EPG) of stool was calculated for each smear, and the geometric mean of EPG was calculated for infected individuals.

Statistical analysis

Survey data were double entered into Microsoft Excel spreadsheet and cross-checked for errors. Data were processed and analysed using Stata/SE version 13.1 for Windows (StataCorp, TX, USA). Differences in proportions were tested using Chi-squared test or the Fisher's exact test, while comparison for continues variables were made using t-test. 95% confidence intervals (95% CI) were estimated to provide uncertainty surrounding the point estimates. Univariate logistic regression was performed to identify risk factors for the outcome of *O. viverrini* infection as determined by Kato-Katz thick smear. Odds ratios (OR) and 95% CI were also computed for the explanatory variables. All variables with a $P < 0.1$ from a likelihood ratio test in univariate analyses were entered into a multivariate logistic regression model and stepwise backwards elimination was used to identify the main risk factors for infection. A $P < 0.05$ was considered statistically significant.

RESULTS

Characteristic of study population at screening

A total of 495 participants, 245 from Sakon Nakhon Province and 250 from Nakhon Phanom Province, were enrolled in the study. The ages of the participants ranged from 15 to 87 years with a median age of 52 years. Except gender, marital status, use of praziquantel and prior information of *O. viverrini* infection, all other personal characteristics of the study participants varied significantly between the two provinces (Table 1). Participants in Nakhon Phanom were slightly younger compared to participants from Sakon Nakhon (mean 47.8 vs 51.2 years). In Nakhon Phanom, a slight majority had primary education (74.0% [95% confidence interval, 95% CI 68.1 – 79.3] vs 72.2% [95% CI 66.1 – 77.8]) and work as farmer (65.6% [95% CI 59.4 – 71.5] vs 62.0% [95% CI 55.6 – 68.1]). Participants from Nakhon Phanom had lower alcohol intake but were more often living with domestic animals i.e. dogs. When considering factors more directly related to helminth infections, more participants in Nakhon Phanom were reported to have consumed raw fish in the

Table 1. Socio-demographic characteristic of the participants in Sakon Nakhon (N=245) and Nakhon Phanom (N=250), Thailand

Characteristic	Category	Overall n (%)	Sakhon Nakhon n (%)	Nakhon Phanom n (%)	*P* value[a]
Gender	Male	218 (44)	116 (47.4)	102 (40.8)	0.145
	Female	277 (56)	129 (52.6)	148 (59.2)	
Age (years, mean±SD)[b]		49.5 ± 12.8	51.2 ± 14.8	47.8 ± 10.3	**0.004**
Age group (years)	<35	77 (15.7)	40 (16.6)	37 (14.8)	**<0.001**
	35 - 45	94 (19.1)	38 (15.8)	56 (22.4)	
	46 - 55	142 (28.9)	55 (22.8)	87 (34.8)	
	>55	178 (36.3)	108 (44.8)	70 (28)	
Education level	No formal schooling	14 (2.8)	3 (1.2)	11 (4.4)	**0.006**
	Primary school	362 (73.1)	177 (72.2)	185 (74)	
	Secondary school	104 (21)	59 (24.1)	45 (18)	
	University	15 (3)	6 (2.5)	9 (3.6)	
Marital status	Single	66 (13.3)	34 (13.9)	32 (12.8)	0.936
	Marriage	351 (70.9)	173 (70.6)	178 (71.2)	
	Widow/separated	78 (15.8)	38 (15.5)	40 (16)	
Occupation	Unemployed/No work	51 (10.3)	(18) (7.4)	33 (13.2)	**0.017**
	Government employee	5 (1)	(3) (1.2)	2 (0.8)	
	Trader	24 (4.9)	(10) (4.1)	14 (5.6)	
	Labourer	99 (20)	(62) (25.3)	37 (14.8)	
	Farmer	316 (63.8)	(152) (62)	164 (65.6)	
Income (Baht/month, mean±SD)		5093±4466	2106±177	8020±242	**<0.001**
Income level (Baht/month)	Low (≤8000)	359 (72.5)	230 (93.9)	129 (51.6)	**<0.001**
	Medium (8001-15000)	128 (25.9)	14 (5.7)	114 (45.6)	
	High (>15000)	8 (1.6)	1 (0.4)	7 (2.8)	
Alcohol consumption	No	281 (56.8)	126 (51.4)	155 (62)	**0.019**
	Yes	214 (43.2)	119 (48.6)	95 (38)	
Domestic animals present	No	114 (23)	107 (43.7)	7 (2.8)	**<0.001**
	Yes	381 (77)	138 (56.3)	243 (97.2)	
Type of domestic animals present	Cat	5 (1.3)	3 (2.2)	2 (0.8)	**<0.001**
	Chicken	11 (2.9)	11 (7.9)	1 (0.4)	
	Dog	365 (95.8)	124 (89.9)	240 (98.8)	
Raw fish consumption	No	114 (23)	113 (46.1)	1 (0.4)	**<0.001**
	Yes	381 (77)	132 (53.9)	249 (99.6)	
Stool examination	No	277 (56)	150 (61.2)	127 (50.8)	**0.024**
	Yes	218 (44)	95 (38.8)	123 (49.2)	
Positive helminthes	No	442 (89.3)	221 (90.2)	221 (88.4)	0.563
	Yes	53 (10.7)	24 (9.8)	29 (11.6)	
Praziquantel used	No	334 (67.5)	174 (71)	160 (64)	0.103
	Yes	161 (32.5)	71 (29)	90 (36)	
Information of *O. viverrini* infection	No	8 (1.6)	8 (3.3)	0 (0)	**0.003**
	Yes	487 (98.4)	237 (96.7)	250 (100)	

SD, standard deviation. [a] Chi-squared or Fisher's exact tests between Sakon Nakhon and Nakhon Phanom. [b] Four individuals have no age recorded.

past (P <0.001), undertaken previous stool examination (P = 0.024), and received information regarding the infection (P = 0.003), when compared to participants in Sakon Nakhon. Finally, among the 381 individuals reported of eating raw fish, the proportion of raw fish consumption were highest in >55 age group (35.3% [95% CI 30.5 – 40.3]), attended primary education (74.3% [95% CI 69.6 – 78.6]) and worked as farmer (67.5% [95% CI 62.5 – 72.1]).

Prevalence and distribution of *O. viverrini*

The overall *O. viverrini* prevalence in the study sample was 15.4% (76/495) and significantly higher in Nakhon Phanom (19.6% [95% CI 14.9 – 25.1]) than in Sakon Nakhon (11.0% [95% CI 7.3 – 15.6] (P = 0.009) (Fig. 1a). Nevertheless, the geometric mean EPG (ranged from 24 – 1848 geometric mean EPG) among *O. viverrini* positive individuals in both areas were similar. No other intestinal helminths were observed. In general, parasite rate in the overall cohort was not differed by age groups (P = 0.154), but the parasite rate was significantly higher in the <35 group (P = 0.025) and the 35 – 45 group (P = 0.003) in Nakhon Phanom than in Sakon Nakhon (Fig. 1b). Furthermore, in Nakhon Phanom, the prevalence of *O. viverrini* were significantly higher than Sakon Nakhon for females and farmers as well as having dogs as domestic animals (all P<0.05). Similarly, with regards to history of helminth infections, the prevalence differed significantly in Nakhon Phanom than Sakon Nakhon (Table 2); high among those who had previous stool examination (P = 0.038) and used of praziquantel (P = 0.02), but low in those who previously tested positive for other helminthes infections (P = 0.045).

Factors associated with opisthorchiasis occurrence

Comprehensive results for all significant co-variables associated with *O. viverrini* infection in univariate analysis (P <0.05) are provided in Table 3, and these variables were further used to build multivariate models with stepwise forward selection. Final model in multivariate logistic regression showed that only being a labourer (adjusted odd ratio [AOR] 4.7 [95% CI 1.08, 20.38], P = 0.039) and farmer (AOR 5.79 [95% CI 1.45, 31.91], P = 0.015) were significantly associated with a higher risk of *O. viverrini* infections in the overall cohort, when compared to the unemployed group.

DISCUSSION

This study describes data on *O. viverrini* prevalence among 495 individuals living in two provinces in northeastern Thailand along the Nam Kam River Basin. The present study indicates significant local variation in *O. viverrini* prevalence between the two study areas; two northern regions with predominately rural inhabitants. The overall relationship between age and parasite prevalence was similar, albeit showed variation in individuals below 45 years between the study areas. The results also show that the main risk factors associated with *O. viverrini* infection in the two communities were related to individual's occupation as being labourers and farmers.

We examined the cross-sectional data to investigate the current prevalence of *O. viverrini* infection in settlements along the Nam Kam River. Our study showed that the overall *O. viverrini* prevalence was high (i.e. 15.4%) and local variation in prevalence was also observed between the two communities living along the river's course. Our study areas are situated in the northeastern region of Thailand and was previously reported to be the region with the highest frequency of *O. viverrini* infections in the country (Kaewpitoon *et al.*, 2008). The habit and frequency of eating raw or undercooked fish named *Koi pla*, a traditional dish that is commonly found in the north and the northeast of Thailand, therefore, contributed to the higher morbidity rate than other regions. The differences in prevalence between the two communities maybe due to their respective distance to the waterbodies where villagers procure fish for consumption. A recent study has shown that the distance to waterbody had an impact on how villagers source the fish used in raw fish dishes (Ong *et al.*, 2016).

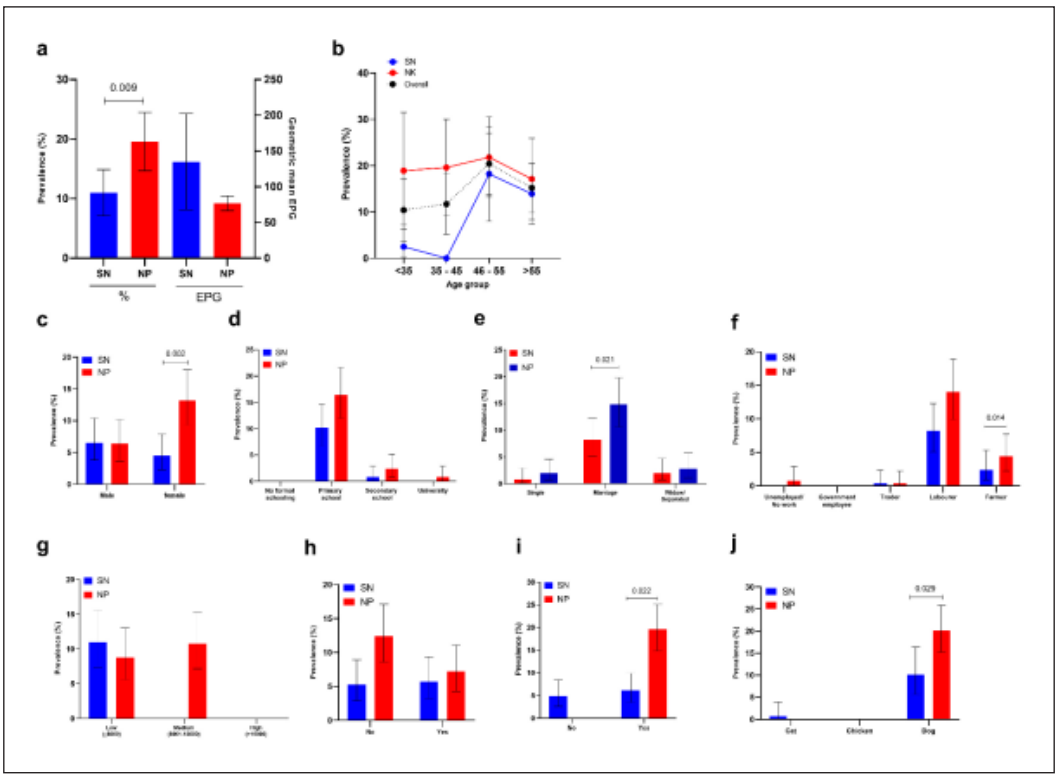

Figure 1. Prevalence of *Opisthorchis viverrini* in Sakon Nakhon (SN; blue) and Nakhon Phanom (NP; red). (a) Area-specific prevalence and geometric mean of eggs per gram faeces (EPG). (b) Age-specific prevalence. (c) Gender-specific prevalence. (d) Prevalence by education level. (e) Prevalence by marital status. (f) Prevalence by type of occupation. (g) Prevalence by income level. (h) Prevalence by alcohol intake. (i) Prevalence by present of domestic animal. (j) Prevalence by type of domestic animal.

Table 2. Past histories of study participants in relation to helminth infections

Characteristic	Category	Sakhon Nakhon (N=245)		*P* value	Nakhon Phanom (N=250)		*P* value
		n	% (95% CI)		n	% (95% CI)	
Eating raw fish	No	10	4.1 (2.0 - 7.4)	0.414	1	0.4 (0.0 - 2.2)	0.196
	Yes	17	6.9 (4.1 - 10.9)		48	19.2 (14.5 - 24.6)	
Previous stool examination	No	16	6.5 (3.8 - 10.4)	0.837	18	7.2 (4.3 - 11.1)	**0.038**
	Yes	11	4.5 (2.3 - 7.9)		31	12.4 (8.6 - 17.1)	
Previous positive helminthes	No	22	9.0 (5.7 - 13.3)	0.159	39	15.6 (11.3 - 20.7)	**0.045**
	Yes	5	2.0 (0.7 - 4.7)		10	4.0 (1.9 - 7.2)	
Praziquantel used	No	16	6.5 (3.8 - 10.4)	0.178	24	9.6 (6.2 - 13.9)	**0.02**
	Yes	11	4.5 (2.3 - 7.9)		25	10.0 (6.7 - 14.4)	
Information of *O. viverrini* infection	No	2	0.8 (0.1 - 2.9)	0.216	0	0	0.999
	Yes	25	10.2 (6.7 - 14.7)		49	19.6 (14.9 - 25.1)	

Table 3. Risk factor analysis for *Opisthorchis viverrini* infection in the Nam Kam River basin: univariate and multivariate adjusted analyses using survey logistic regression (n = 76)

Characteristic	Category	Cases		Prevalence (%) (95% CI)	Unadjusted OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
		n	N					
Total		76	419	15.4 (12.3 - 18.8)				
Area	Sakhon Nakhon	27	245	11.0 (7.4 - 15.6)	1.00		1.00	
	Nakhon Phanom	49	250	19.6 (14.9 - 25.1)	1.97 (1.19 - 3.27)	**0.009**	1.90 (0.93 - 3.89)	0.08
Occupation	Unemployed/No work	2	51	3.9 (0.5 - 13.5)	1.00		1.00	
	Government employee	0	5	0	-		-	
	Trader	2	24	8.3 (1.0 - 27.0)	2.23 (0.29 - 16.85)	0.438	2.46 (0.30 - 20.08)	0.4
	Labourer	55	316	17.4 (13.4 - 22.0)	5.16 (1.22 - 21.87)	**0.026**	4.70 (1.08 - 20.38)	**0.039**
	Farmer	17	99	17.2 (10.3 - 26.1)	5.08 (1.13 - 22.93)	**0.035**	6.79 (1.45 - 31.91)	**0.015**
Income level (Baht/month)	Low (≤8000)	49	359	13.7 (10.3 - 17.6)	1.00		1.00	
	Medium (8001-15000)	27	128	21.1 (14.4 - 29.2)	1.69 (1.00 - 2.85)	**0.048**	1.18 (0.62 - 2.25)	0.609
	High (>15000)	0	8	0	-		-	
Previous stool examination	No	34	277	12.3 (8.7 - 16.7)	1.00		1.00	
	Yes	42	218	19.3 (14.3 - 25.1)	1.71 (1.04 - 2.79)	**0.033**	1.02 (0.56 - 1.86)	0.936
Previous positive helminthes	No	61	442	13.8 (10.7 - 17.4)	1.00		1.00	
	Yes	15	53	28.3 (16.8 - 42.3)	2.47 (1.28 - 4.75)	**0.007**	1.46 (0.67 - 3.17)	0.339
Praziquantel used	No	40	334	12.0 (8.7 - 15.9)	1.00		1.00	
	Yes	36	161	22.4 (16.2 - 29.6)	2.12 (1.29 - 3.48)	**0.003**	1.72 (0.93 - 3.16)	0.084

Differences in the infection levels in fish are also dependent on the waterbody, thus can affect the risk of human exposure to the infection (Ong *et al*., 2016). The strikingly high consumption of raw fish in Nakhon Phanom (i.e. 99.6%) may also contribute to the higher prevalence of *O. viverrini* in this area. Our direct observation revealed that this environmental condition may contribute to the high *O. viverrini* infection rate among the Nakhon Phanom population: the population lives along the Nam Kam River and its streams with a large fish stock; the abundance of freshwater fish leads to the regular consumption of raw fish. This phenomenon has been explained by a common process originating from the unidirectional river flow that favours the displacement and downstream dispersion of fish (Blasco-Costa *et al*., 2013). Thus, the unidirectional river flow supported the existence of a longitudinal gradient in trematode abundance, which increases from downstream-to-upstream along the river. Nevertheless, our study did not examine possible environmental influences such as proximity to different kinds of waterbodies in relation to the infection. Therefore, comparative multifactorial studies are necessary to gain more insight into the relationship between this environmental factor and the prevalence of *O. viverrini* infection in this region.

The prevalence of *O. viverrini* infection gradually increases in an age-dependent manner and reaching a plateau among the elderly. A similar age-specific pattern has been observed for *O. viverrini* infection in other parts of Thailand (Sithithaworn & Haswell-Elkins, 2003; Kaewpitoon *et al*., 2008; Chudthaisong *et al*., 2015), Laos (Sayasone *et al*., 2007; Sripa *et al*., 2011; Forrer *et al*., 2012) and Cambodia (Yong *et al*., 2012). The overall prevalence of *O. viverrini* in individuals less than 45 years old was considerably lower than their older counterparts, furthermore, Nakhon Phanom has a higher prevalence in all age groups than Sakon Nakhon. One reason for a higher infection prevalence among the older age groups in Nakhon Phanom is likely to be their socializing behavior – actively involved in social gathering where raw fish dishes are often available. Due to its location on the Thailand-Laos international border, Nakhon Phanom is home to a large number of young ethnic Laotians seeking job opportunities, who may have different eating habits than the locals. Moreover, the consumption of raw fish dishes is part of one's social obligation and integration among Laotian subcultures (Xayaseng *et al*., 2013). Despite the little difference in prevalence among the age groups in our study, people at all ages are at risk of being infected with *O. viverrini*. CCA is an *Opisthorchis*-associated cancer which can take 30 – 40 years to develop, people infected with *O. viverrini* in their early age may only be diagnosed with this cancer during their most productive years of their life, with far reaching negative consequences for their families and the entire communities (Sriamporn *et al*., 2004; Andrews *et al*., 2008; Steinmann *et al*., 2011). The interrelation of *O. viverrini* infection and age becomes a serious public health issue and requires intergenerational (i.e. between children and their parents) and transgenerational (i.e. across multiple generations) approaches in designing health education campaigns, such as delivering tailored health messages and measures to each specific age group (Phongluxa *et al*., 2013).

In the overall cohort, there is clear evidence of infection prevalence being associated with low socio-economic status within the community. Our study has shown that the prevalence of *O. viverrini* were significantly higher among farmers (Fig. 1f) in Nakhon Phanom than in Sakon Nakhon and working as a labourer or a farmer were both independently associated with increased odds of *O. viverrini* infections, thus highlighting the importance of both individual and community level poverties. This finding is consistent with the mounting evidence from other parts of the country that suggest people with lower socio-economic status have higher risk to the infection (Ong *et al*., 2016; Kim *et al*., 2017; Prakobwong *et al*., 2017). In previous studies, farmers were found to have higher infection prevalence than non-farmers (Forrer *et al*., 2012; Kaewpitoon *et al*., 2012), suggesting that the observed higher infection

rate in people who are farmers is occupationally associated. Furthermore, higher percentage of participants who were farmers (67.5%) claimed to have consumed raw fish than participants with other occupations in the study areas. This is because farmers in our study areas often harvest fish from Nam Kam River or from their rice fields and then prepare and consume the catches on the spot. Agricultural labourers may also consume raw fish in our study areas from the popular dish of *Koi pla* due to its ease of preparation, wide distribution and relatively cheap price. In addition, most of the labourers in the study areas come from the neighboring country, and the finding that parasitic infections are prevalent among these labourers demonstrate the need for targeted control measures in foreign labourers working in Thailand. Nevertheless, no clear pattern was observed between infection rates and the disclosure of income. Thus, we believe that the reasons for the higher risk of *O. viverrini* infections in socio-economically deprived groups (i.e. farmer and labourer) in the study areas may be centered around the lifestyle compounded with poverty in this region.

This study has several limitations. First, while the convenience sampling approach used in this study was efficient and cost-effective, it has an inherent selection bias. Our survey was conducted on a house-to-house basis during the weekdays, meaning that only older adults (i.e. above 46 years) were disproportionally represented (Table 1). Over-representation of this age group among our samples may likely overestimate the true parasite prevalence in the study areas. Second, due to the imperfect sensitivity of the Kato-Katz technique, the true *O. viverrini* prevalence maybe considerably higher than the rate reported here (Johansen *et al.*, 2010). Third, our study has undersampled younger population that could have led to an underestimation of the overall parasite prevalence. Many adolescents in both study areas are in school or refused to be sampled when our survey was held. *Opisthorchis* infection in this younger age group is not well characterised and warrants further investigation. Fourth, the survey design did not permit georeferencing of individual household locations and the complete climate data from each study areas. Therefore, it was not possible to use spatiotemporal analysis to investigate patterns within individual sites. A recent spatiotemporal study in the same region has highlighted the highly dynamic nature of *O. viverrini* transmission, together with intensified ecological process modulated by human and environmental factors (Kim *et al.*, 2016). As such, investigation on the spatial and temporal variations in our study area, along with social and ecological understandings of the parasite transmission dynamics will be needed to provide additional information for future interventions planning.

In conclusion, this study provides new insight into the distribution of *O. viverinni* infection in Sakon Nakhon and Nakhon Phanom provinces. It highlights *O. viverrini* infection as a significant endemic disease with potential health problems in the water-based communities along the Nam Kam River. The high prevalence of *O. viverrini* infection in these areas, particularly in Nakhon Phanom Province, is alarming and warrants urgent public health intervention. In Nakhon Phanom Province, raw fish consumption is common and *O. viverrini* prevalence is higher than in Sakon Nakhon. Further, socio-economically deprived groups, namely labourers and farmers, are associated with opisthorchiasis occurrence. This implies a potential benefit to direct additional *O. viverrini* control activities to labourers and farmers, such as improving access to diagnosis and treatment. Overall, this study calls for the local authorities and communities in the northeastern provinces of Thailand to integrate their actions and address *O. viverrini* infection by building awareness and strengthening knowledge and health practices on the infection.

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Conflict of Interests

The authors declare no competing financial interests.

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