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Current prevalence of *Opisthorchis viverrini* infection and associated risk factors in Nakhon Phanom Province, Northeastern Thailand

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Abstract. Opisthorchiasis caused by *Opisthorchis viverrini* infection is a major public health concern in Thailand. Despite many decades of national campaigns in place to reduce and control opisthorchiasis in Thailand, the infections remain to exist particularly in the northern and northeastern parts of the country. This study aimed to evaluate the current prevalence of *O. viverrini* infection in rural communities in northeast Thailand. A cross-sectional survey was conducted between February and October 2018 in three districts (Na Kae, That Phanom and Wang Yang) in Nakhon Phanom Province, Thailand. Demographic data were collected using a standardised questionnaire. Stool samples were collected and processed using the Kato-Katz technique to determine the presence of *O. viverrini* and other intestinal parasites. In total, 564 individuals were enrolled. The overall intestinal helminth infections were 15.2% (95% CI: 12.4–18.5). Species distribution included a majority of *O. viverrini* mono-infections (12.9%), followed by *Strongyloides stercoralis* (1.4%) and *Taenia* spp. (0.4%). The prevalence of *O. viverrini* was significantly higher in Wang Yang district (P = 0.022), in males (P = 0.004) and those previously positive with helminth infections (P<0.001) and received treatment of anti-helminths (P<0.001), than in their counterparts. Multivariate regression analysis revealed that being male (adjusted odds ratio [aOR] 1.77, P = 0.035) and those who previously tested positive for helminth infections (aOR 8.69, P<0.001) were significantly associated with a higher odd of *O. viverrini* infections, but lower in those who had previous stool examination (aOR 0.22, P = 0.001). This study demonstrated that the updated prevalence of *O. viverrini* infection is still high in rural communities in northeast Thailand. The data of this study will be useful to guide and improve strategies for future *O. viverrini* and other helminths prevention and control in this region.

INTRODUCTION

The incidence of neglected tropical diseases caused by intestinal parasites is an increasingly serious global health issue. A quarter of the world population suffers from at least one type of intestinal parasitic infection with approximately 450 million individuals believed to be a host for multiple parasitic species (Robertson *et al.*, 2013). Southeast Asian countries such as Myanmar, Vietnam and Laos do not escape from the wrath of these parasites, with Thailand being one of the most affected, particularly in the north-eastern region (Conlan *et al.*, 2012; Songserm *et al.*, 2012; Tun *et al.*, 2013).

Among these intestinal parasites, the helminth *Opisthorchis viverrini*, or commonly known as the liver fluke, does seem to have a strong foothold in this region.

An estimate of 8 to 10 million people in Thailand is infected with *O. viverrini* (Sithithaworn *et al.*, 2012), with the north-eastern region recording a higher prevalence when compared with the neighbouring provinces (Suwannatrai *et al.*, 2018). *O. viverrini*, which was classified as Class 1 carcinogen by The International Agency for Research on Cancer (IARC) in 2009, is commonly associated with incidence of hepatobiliary diseases and the causative agents of bile duct cancer (cholangiocarcinoma) (Sripa *et al.*, 2007; Bouvard *et al.*, 2009; Laoraksawong *et al.*, 2018). Infections by these intestinal helminths are rarely diagnosed at an early stage of exposure due to its asymptotic nature, exasperating the prevalence of opisthorchiasis in these regions (Kaewpitoon *et al.*, 2018).

A continuous effort has been undertaken by the Ministry of Public Health to eradicated the persistent *O. viverrini* infection among the rural population in Thailand, such as the Eco-Health/One Health approach (Sripa *et al.*, 2015) and the Cholangiocarcinoma Screening and Care Programme (Khuntikeo *et al.*, 2015). These programmes have had significant success, bringing down the national average infection rate to 9.4% in 2000, further dropping to 8.7% in 2009. However, the disease remains prevalent, especially in the north and north-eastern regions, being among the highest affected areas in the world (Sithithaworn *et al.*, 2012).

Among the 20 provinces in Northeastern Thailand, Nakhon Phanom Province has consistently shown a high incidence of infection by these helminths (Thaewnongiew *et al.*, 2014). Studies have shown an infection rate as high as 20% among communities in this province, with a substantial intensity of *O. viverrini* present among the cyprinid fish found in this region (Sithithaworn *et al.*, 2012; Chuangchaiya *et al.*, 2019). Through this study, we aimed to evaluate the current prevalence of *O. viverrini* infection in rural communities in Northeastern Thailand. Based on the data obtained through this cross-sectional study, we also sought to identify associated risk factors linked with the prevalence of *O. viverrini* present in the Nakhon Phanom Province.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ministry of Health of Thailand (reference no. 5/2560) and the Ethics Review Committee of the Sawang Dan Din Crown Prince Hospital (reference no. SWDCPH 2017-009). Informed consent was obtained from all the participants before they were enrolled in the study.

Study area and sample population

The study was carried out between February and October 2018 in three districts in Nakhon Phanom Province, Thailand namely Na Kae, That Phanom and Wang Yang (Figure 1). The three districts have a combined population of 175,515 people (Department of Provincial Administration, Thailand). The sample size for study participants was calculated using the following formula the Cochran's 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 40.9% for Nakhon Phanom (Thaewnongiew *et al.*, 2014) and e is the allowed error margin which is set to 5%. In addition, contingencies were adjusted by adding another 40% of individuals, giving us a minimum of 519 participants to be sampled.

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 the selected survey point for study participation. Enrolled participants were interviewed using standardised questionnaires in Thai language 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 history of behavioural aspects (i.e. raw fish consumption, type of fish consumption [*Hampala dispar*, *Osteochilus vittatus*, *Barbonymus gonionotus* and *Anematichthys apogon*], open defecation, previous stool examination, the positivity of helminth infection, anthelminthic drug intake and prior knowledge about *O. viverrini* infection). All

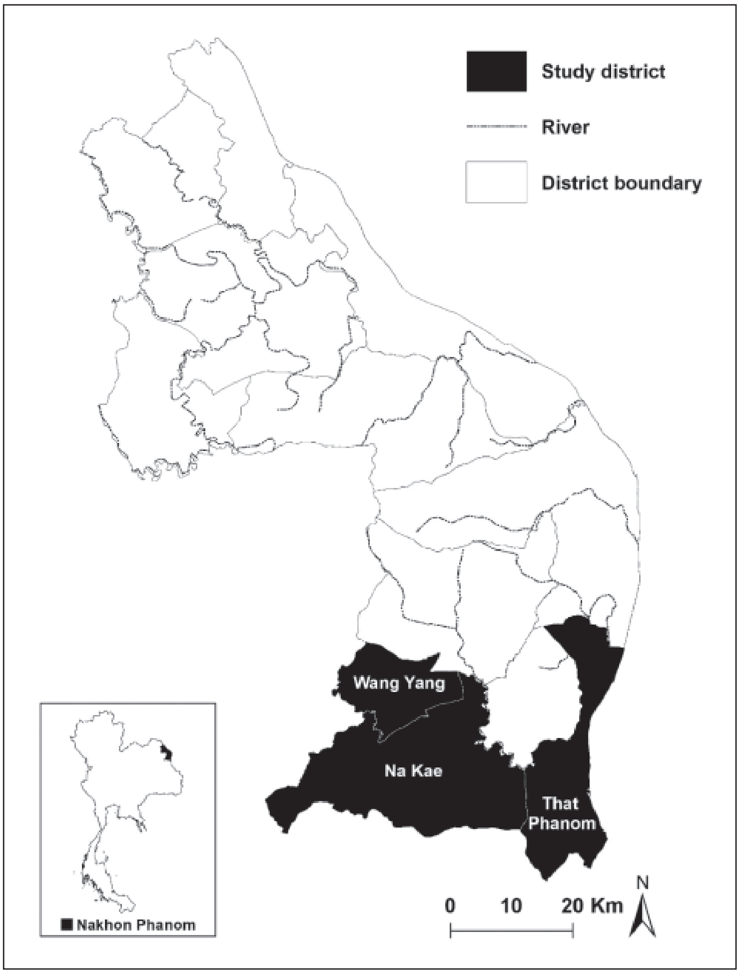

Figure 1. Map showing the three study districts in Nakhon Phanom Province, Thailand.

completed questionnaires were checked for accuracy and completeness.

Sample collection and parasitological examination

Study participants were invited to provide a single stool sample. Inclusion criteria were individuals who willing to participate in the study, aged ≥18 years old and living in the area for at least six months. After proper instruction, a biohazard zipper bag containing stool container and spatula, labelled 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.

Modified thick smears (Chuangchaiya *et al.*, 2019) were prepared after the stool collection using a commercially available Kato-Katz Kit (Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand). 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.

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. 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 $P<0.05$ 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

Study population and prevalence
A total of 564 participants (representing approximately 0.3% of the combined population in the three districts) were screened for intestinal helminth infections out of which 86 (15.2%; 95% CI: 12.4 – 18.5) were positive (Table 1). Species distribution included a majority of *O. viverrini* mono-infections (n = 73) [12.9%; 95% CI: 10.3 – 15.9], followed by *S. stercoralis* (n = 8) [1.4%; 95% CI: 0.6 – 2.8] and *Taenia* spp. (n = 2) [0.4%; 95% CI: 0.0 – 1.3]. Furthermore, three cases of double co-infections were observed comprising two cases of *O. viverrini*/*S. stercoralis* (0.4%; 95% CI: 0.0 – 1.3) and one case of *O. viverrini*/*Taenia* spp. (0.2%; 95% CI: 0.0 – 1.0).

The socio-demographic of the participants and the specific prevalence of *O. viverrini* is summarized in Table 2. Briefly, the overall prevalence differed significantly ($P = 0.022$) by district: highest in Wang Yang, followed by That Phanom and lowest Na Kae. The prevalence of *O. viverrini* also differed significantly ($P = 0.004$) in male than in female participants. Similarly, with regards to the history of helminth infections, the prevalence differed significantly among those who previously tested positive for other helminth infections ($P<0.001$) and previously used of anti-helminths ($P<0.001$).

Table 1. Prevalence of intestinal helminth infections among population (N=564) living in the three districts in Nakhon Phanom Province, Thailand

Intestinal parasitic infection	n (%)	95% CI
Overall infection	86 (15.2)	12.4–18.5
Opisthorchis viverrini	73 (12.9)	10.3–15.9
Strongyloides stercoralis	8 (1.4)	0.6–2.8
Taenia spp.	2 (0.4)	0.0–1.3
O. viverrini + *S. stercoralis*	2 (0.4)	0.0–1.3
O. viverrini + *Taenia* spp.	1 (0.2)	0.0–1.0

n: Number of positive sample; CI: Confidence interval.

Table 2. Socio-demographic of the participants and the specific prevalence of *Opisthorchis viverrini* in Nakhon Phanom Province, Thailand

Characteristics	Category	Sample examined, n (%)	Sample positive, n (%)	*P* value[a]
District	Na Kae	178 (31.6)	17 (9.6)	0.022*
	That Phanom	202 (35.8)	24 (11.9)	
	Wang Yang	184 (32.6)	35 (19.0)	
Subdistrict	Kok Sawang	102 (18.1)	4 (3.9)	<0.001*
	Na Nat	100 (17.7)	20 (20)	
	Nong Sang	178 (31.6)	17 (9.6)	
	Yot Chat	184 (32.6)	35 (19.0)	
Gender[c]	Male	248 (44.0)	45 (18.2)	0.004*[b]
	Female	316 (56.0)	31 (9.8)	

Table 2 continued...

Characteristics	Category	Sample examined, n (%)	Sample positive, n (%)	*P* value[a]
Age group[c]	18–29	23 (4.1)	0 (0.0)	0.111
	30–39	58 (10.4)	10 (17.2)	
	40–49	155 (27.7)	27 (17.4)	
	50–59	196 (35.0)	26 (13.3)	
	≥60	128 (22.8)	13 (10.2)	
Education level	No school	12 (2.1)	0 (0.0)	0.051
	Primary	439 (77.8)	63 (14.4)	
	Secondry	90 (16.0)	7 (7.8)	
	Tertiary	23 (4.1)	6 (26.1)	
Marital status[c]	Single	45 (8.0)	5 (11.1)	0.838
	Married	467 (83.4)	65 (13.9)	
	divorced/widowed	49 (8.7)	6 (12.2)	
Household montly income[c]	No income	226 (40.2)	25 (11.1)	0.376
	≤THB 8000	310 (55.2)	47 (15.2)	
	>THB 8001	26 (4.6)	4 (15.4)	
Occupation[c]	Farmer/Agriculturist	450 (79.9)	62 (13.8)	0.344
	Labour	77 (13.7)	12 (15.6)	
	Housekeeper	19 (3.4)	0 (0.0)	
	Others	17 (3.0)	2 (11.8)	
History of eating raw fish	No	177 (31.4)	20 (11.3)	0.353[b]
	Yes	387 (68.6)	56 (14.5)	
History of faeces examination	No	223 (39.5)	26 (11.7)	0.377[b]
	Yes	341 (60.5)	50 (14.7)	
History of positive helminth	No	440 (78.0)	30 (6.8)	<0.001*[b]
	Yes	124 (22.0)	46 (37.1)	
History of taking anti-helminths	No	352 (62.4)	31 (8.8)	<0.001*[b]
	Yes	212 (37.6)	45 (21.2)	
Consume alcohol	No	318 (56.4)	37 (11.6)	0.171[b]
	Yes	246 (43.6)	39 (15.9)	
Having domestic animal	No	255 (45.2)	27 (10.6)	0.083[b]
	Yes	309 (54.8)	49 (15.9)	
Type of animal[c]	No animal	255 (46.4)	27 (10.6)	0.089
	Dog	265 (48.2)	46 (17.4)	
	Cat	10 (1.8)	1 (10.0)	
	Cat and dog	20 (3.6)	1 (5.0)	
Defecate on water	No	229 (40.6)	27 (11.8)	0.380[b]
	Yes	335 (59.4)	49 (14.6)	
Hampala dispar[c]	No	326 (59.3)	49 (15.0)	0.379[b]
	Yes	224 (40.7)	27 (12.1)	
Osteochilus vittatus[c]	No	377 (68.6)	58 (15.4)	0.143[b]
	Yes	173 (31.4)	18 (10.4)	
Barbonymus gonionotus[c]	No	266 (48.4)	42 (15.8)	0.217[b]
	Yes	284 (51.6)	34 (12.0)	
Anematichthys apogon[c]	No	468 (85.1)	67 (14.3)	0.491[b]
	Yes	82 (14.9)	9 (11.0)	
Received info on *Opisthorchis*	No	19 (3.4)	2 (10.5)	0.999[b]
	Yes	545 (96.6)	74 (13.6)	

[a]The *P* values for differences in positivity rates between subcategory were calculated based on Pearson's chi-square test.
[b]Fisher's exact test.
[c]Missing data for gender (n=4), age group (n=4), marital status (n=3), income level (n=2), occupation (n=1), type of animal (n=14), *H. dispar* (n=14), *O. vittatus* (n=14), *B. gonionotus* (n=14) and *A. apogon* (n=14).
*Significant difference $P<0.05$. THB: Thai Bhat.

Factor 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. The final model in multivariate logistic regression showed that being male (adjusted odds ratio [aOR] 1.77 [95% CI: 1.04 – 2.99], P = 0.035) and tested positive for helminth infections (aOR 8.69 [95% CI: 4.32 – 17.48], $P<0.001$) were significantly associated with higher odds of contracting an *O. viverrini* infection. Nonetheless, the odds of *O. viverrini* infection was significantly lower in those who had previous stool examination (aOR 0.22 [95% CI: 0.10 – 0.51], P = 0.001), when compared to those who had not undergone a stool examination.

DISCUSSION

Although effective anti-helminth drugs and prevention control programmes are in place, intestinal helminth infections remain a critical issue for the rural communities in Thailand. The risk factors commonly associated with these diseases, especially concerning *O. viverrini*, was evaluated in the selected rural population in Nakhon Phanom Province in upper Northeastern Thailand to obtain a better understanding of the underlying reasons behind the tenacious parasitic infections here. This study demonstrated that *O. viverrini* appears to be the highest contributing parasite to the total number of positive helminth infections, followed at a distance by *S. stercoralis* and *Taenia* spp. A similar pattern of prominence favouring *O. viverrini* over *S. stercoralis* and *Taenia* spp. was also observed in several studies, including by Kaneshiro *et al.* (2019) that was conducted in Laos; highest for *O. viverrini* (16.5%), followed by *S. stercoralis* (10.8%) and *Taenia* spp. (3.3%). Interestingly, it is not uncommon for communities in Northeastern Thailand and surrounding countries to suffer from double infection by both *O. viverrini* and *S. stercoralis* at the same time (Nontasut *et al.*, 2005; Kitvatanachai *et al.*, 2008; Sayasone *et al.*, 2009; Janwan *et al.*, 2011; Kaewpitoon *et al.*, 2019), further highlighting the prevalence of these parasitic infections.

The present study managed to show a significant proportion of *O. viverrini* infection among male respondents. This result was consistent with previous findings where males were more likely to have a higher infection rate when compared with their female counterparts (Boonjaraspinyo *et al.*, 2013; Laoraksawong *et al.*, 2018; Chuangchaiya *et al.*, 2019). The disparity in infection distribution among the genders may be attributed to behavioural factors and socialization patterns that have men consuming more raw fish than females, especially among the rural communities in Thailand (Kaewpitoon *et al.*, 2012; Songserm *et al.*, 2012). Among these habits, consumption of raw or undercooked fish has been attributed as a main contributing factor to the prevalence of *O. viverrini* in the country (Thaewnongiew *et al.*, 2014); this is especially true among those involved in farming and labour-intensive occupation which are typically male-dominated. Convenience, a lack of proper cooking facilities and traditional preparations make it more likely for the fishes to be consumed in raw form, causing an increased risk towards *O. viverrini* infection.

Our study showed that local variation in prevalence was observed between the three districts in Nakhon Phanom Province: highest in Wang Yang, followed by That Phanom and lowest Na Kae. The differences in prevalence between the districts maybe due to their respective distance to the waterbodies where communities procure fish for consumption. A study by Ong *et al.* (2016) has shown that the distance to waterbody had an impact on how villagers source the fish used in raw fish dishes. Differences in the infections levels in fish are also dependent on the waterbody, thus can affect the risk of human exposure to the infections (Ong *et al.*, 2016). Compare to That Phanom and Na Kae, Wang Yang district is located within the waterway of the Nam Kam River which flows from the neighbouring province of Sakon Nakhon

Table 3. Univariate and multivariate analysis of risk factors for acquiring *Opisthorchis viverrini* infection in Nakhon Phanom Province, Thailand

Characteristic	Category	n/N	Unadjusted OR (95% CI)	*P* value	Adjusted OR† (95% CI)	*P* value
District	Na Kae	17/178	1.00		1.00	
	That Phanom	24/202	1.28 (0.66–2.46)	0.466	0.62 (0.30–1.25)	0.179
	Wang Yang	35/184	2.22 (1.20–4.14)	0.012*	1.42 (0.71–2.85)	0.320
Gender	Male	45/248	2.04 (1.25–3.33)	0.005*	1 77 (1.04–2.99)	0.035*
	Female	31/316	1.00		1.00	
Age group	18-29	0/23	na	na	na	na
	30-39	10/58	1.84 (0.76–4.49)	0.178	2.32 (0.83–6.46)	0.109
	40-49	27/155	1.87 (0.92–3.79)	0.084	1.73 (0.79–3.75)	0.164
	50-59	26/196	1.35 (0.68–2.74)	0.402	1.16 (0.54–2.50)	0.699
	≥60	13/128	1.00		1.00	
Education level	No school	0/12	na	na	na	na
	Primary	63/439	1.99 (0.88–4.49)	0.099	2.27 (0.94–5.49)	0.068
	Secondary	7/90	1.00		1.00	
	Tertiary	6/23	4.18 (1.25–14.02)	0.020*	2.91 (0.77–10.94)	0.114
Marital status	Single	5/45	1.00		1.00	
	Married	65/467	1.29 (0.49–3.39)	0.601	1.08 (0.38–3.13)	0.882
	divorced/widowed	6/49	1.12 (0.32–3.94)	0.864	0.92 (0.23–3.68)	0.902
Household monthly income	No income	25/226	0.68 (0.22–2.15)	0.515	0.76 (0.21–2.78)	0.682
	≤THB 8000	47/310	0.98 (0.32–2.98)	0.976	0.75 (0.22–2.67)	0.667
	>THB 8001	4/26	1.00		1.00	
Occupation	Farmer/Agriculturist	62/450	1.20 (0.27–5.37)	0.813	1.86 (0.31–11.26)	0.501
	Labour	12/77	1.38 (0.28–6.85)	0.690	2.23 (0.35–13.98)	0.394
	Housekeeper	0/19	na	na	na	na
	Others	2/17	1.00		1.00	
History of eating raw fish	No	20/177	1.00		1.00	
	Yes	56/387	1.33 (0.77–2.29)	0.307	1.06 (0.58–1.93)	0.853
History of faeces examination	No	26/223	1.00		1.00	
	Yes	50/341	1.30 (0.78–2.16)	0.308	0.22 (0.10–0.51)	0.001*
History of positive helminth	No	30/440	1.00		1.00	
	Yes	46/124	8.06 (4.79–13.56)	<0.001*	8.69 (4.32–17.48)	<0.001*

Table 3 continued...

Characteristic	Category	n/N	Unadjusted OR (95% CI)	*P* value	Adjusted OR[†] (95% CI)	*P* value
History of taking anti-helminths	No	31/352	1.00		1.00	
	Yes	45/212	2.79 (1.70–4.57)	<0.001*	0.75 (0.37–1.52)	0.423
Consume alcohol	No	37/318	1.00		1.00	
	Yes	39/246	1.43 (0.88–2.32)	0.147	1.04 (0.58–1.85)	0.906
Having domestic animal	No	27/255	1.00		1.00	
	Yes	409/309	1.59 (0.96–2.63)	0.070	1.56 (0.89–2.71)	0.114
Type of animal	No animal	27/255	2.25 (0.29–17.5)	0.438	2.16 (0.25–18.97)	0.487
	Dog	46/265	3.99 (0.52–30.56)	0.183	3.99 (0.46–34.58)	0.208
	Cat	1/10	2.11 (0.12–37.72)	0.611	1.26 (0.06–27.12)	0.872
	Cat and dog	1/20	1.00		1.00	
Defecate on water	No	27/229	1.00		1.00	
	Yes	49/335	1.28 (0.78–2.12)	0.333	1.0 (0.57–1.75)	0.997
Hampala dispar	No	49/326	0.77 (0.47–1.28)	0.321	1.12 (0.64–1.94)	0.674
	Yes	27/224	1.00		1.00	
Osteochilus vittatus	No	58/377	0.64 (0.36–1.12)	0.118	1.65 (0.90–3.04)	0.104
	Yes	18/173	1.00		1.00	
Barbonymus gonionotus	No	42/266	0.73 (0.45–1.18)	0.196	1.26 (0.74–2.15)	0.395
	Yes	34/284	1.00		1.00	
Anematichthys apogon	No	67/468	0.74 (0.35–1.55)	0.420	1.33 (0.59–3.01)	0.479
	Yes	9/82	1.00		1.00	
Received info on *Opisthorchis*	No	2/19	1.00		1.00	
	Yes	74/545	1.34 (0.30–5.89)	0.703	0.80 (0.16–3.95)	0.786

[†]Data were adjusted for district, gender, education level, history of positive helminth and history of taking anti-helminths.
*Significant difference $P<0.05$. Reference value (i.e. 1.00) was based on the lowest proportion of infection in each category.
n: Number of individuals positive for *O. viverrini*, N: Number of individuals tested for *O. viverrini*; na: Not applicable; OR: Odd ratio; CI: Confidence interval; THB: Thai Bhat.

toward downstream of the Mekong River. Our previous study revealed that population lives along the Nam Kam River and its stream with a large freshwater fish stock leads to the regular consumption of raw fish (Chuangchaiya *et al.*, 2019). 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 flows supported the existence of a longitudinal gradient in trematode abundance, which increases from upstream-to-downstream along the river. Nevertheless, our study did not examine possible environmental influences such as proximity to different kinds of waterbodies in the 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 area.

The prevalence of *O. viverrini* infection in the present study was not significantly different among age groups. This is in contrast to previous findings where age-specific patterns have been observed for *O. viverrini* infection on other parts of Thailand (Chudthaisong *et al.*, 2015; Nakbun *et al.*, 2018; Chuangchaiya *et al.*, 2019), Laos (Sripa *et al.*, 2011; Forrer *et al.*, 2012) and Cambodia (Yong *et al.*, 2012). Despite the no difference in prevalence among the age groups in our study, people at all ages are at risk of being infected with *O. viverrini*. Cholangiocarcinoma is an *Opisthorchis*-associated cancer which can take 30-40 years to develop and people infected with *O. viverrini* in their early age may 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 (Andrews *et al.*, 2008; Steinman *et al.*, 2011). The interrelation of *O. viverrini* infection and age becomes a serious public health issue and requires intergenerational and transgenerational approaches in designing health educations campaign, such as delivering tailored health messages and measures to each specific age group (Phongluxa *et al.*, 2013).

About 40% of the study population in the present study had not undergone a stool examination prior to this study, which is a common occurrence in nearby regions (Boonjaraspinyo *et al.*, 2013). This may indicate a dearth of access to public health facilities which are aimed at preventive screening and subsequent treatment that potentially aids in the eradication or minimizing *O. viverrini* infection among this rural community. Incidentally, a multivariate analysis of our data indicates that those who underwent a previous stool examination had lower odds of contracting the parasite. It would be assumed that those who had been tested would also have access to the appropriate treatment protocols should they be positive. This would be consistent with the above-mentioned argument that preventive testing does indeed play a crucial role in diminishing the presence of *O. viverrini* in a population.

A significant prevalence of *O. viverrini* infection was found among the respondent who had been tested positive and underwent anti-helminths treatment protocols. In addition to this, the former was also found to be a significant risk factor for contracting the intestinal parasite, almost nine times more likely than the reverse. Both the above-mentioned factors are closely related, as those who received treatment would have to be diagnosed first. Although only 22% of the study population had been previously infected by intestinal helminths, they make up the larger incidence of positive cases, be it in terms of numbers or percentage of cases. This would suggest a low compliance with completion of prescribed dosage and post-treatment follow-ups as well as maintenance of unhealthy habits, primarily the consumption of raw freshwater fish dishes that led to the initial infection. Several studies have also indicated the readily available anti-helminth drug, praziquantel, may be discouraging patients against permanently avoiding this unhealthy dietary habit; with some actively seeking praziquantel treatment upon consumption of traditional raw fish dishes (Grundy-Warr *et al.*, 2012;

Saengsawang *et al.*, 2016). Despite having a reported cure rate as high as 96 to 100% (Soukhathammavong *et al.*, 2011) there is a common misconception on the protective effect of praziquantel against reinfection, eventually becoming a contributing risk factor towards the infection by *O. viverrini* (Saengsawang *et al.*, 2013). It has been shown that repeated cycle of infection and treatment may end up increasing the risk of developing cholangiocarcinoma (Charoensuk *et al.*, 2011) as a result of free radical release from inflammatory cells (Sudsarn *et al.*, 2016).

Based on the results obtained, it appears that parasitic helminth infections, especially *O. viverrini*, remains a pressing issue despite a concentrated effort by the Thailand government. One of the main reasons attributed to this occurrence is the lack of culturally sensitive and focused health education programmes, particularly on the food consumption practices of the local population (Puangsa-art *et al.*, 2006). Proactive measures encouraging and engaging the public to participate in the government programmes aimed at providing awareness on the danger of risk factors associated with *O. viverrini* infection should be intensified. In addition to this, measures to improve sanitation and personal hygiene do go a long way in reducing the prevalence of the disease. This is based on studies where a multipronged approach, combining anthelmintic therapy, sanitation and effective education can bring about a significant reduction and prevent the occurrence of reinfection (Sornmani *et al.*, 1984; Upatham *et al.*, 1988).

A number of caveats should be considered in this study. 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 mostly during the weekends, meaning that younger adults (i.e. <30 years) were disproportionally represented. This group of individuals typically away from home during weekend in our study area. Under-representation of this age group among our samples may likely underestimate 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.*, 2020). Third, the survey design did not permit geo-referencing of individual household locations and the complete climate data from each study districts. Therefore, it was not possible to use spatiotemporal analysis to investigate patterns within individual sites. Fourth, the validity of the information provided by respondents in the absence of independent checks or attempts to minimize errors of recall. These kinds or research limitations are difficult to avoid in areas where funding is often largely inadequate.

In conclusion, this study has managed to show a persistent of *O. viverrini* infection in the rural communities in Nakhon Phanom Province. It is hoped that the findings in the present study would provide critical data that can be used towards improving current measures in eradicating of the disease. It is important to create prevention campaigns to persuade people not to consume raw food, but to eat fully cooked dished that are both tasty and free from liver fluke infection. For a healthy community, it is imperative to develop healthy eating behaviours and to teach the people how to protect themselves from the liver fluke infection. For children, educating and exchanging proactive learning in teaching are likely to play an important role in controlling transmission. This should help to adjust the consumption behaviour and personal hygiene in the communities. Efforts to overcome known risk factors, such as a lack of prior testing and previous positive helminths diagnosis, would go a long way in providing long-term mitigation measures, ultimately improving health standards of the local rural communities.

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Conflict of interests

The authors declare no competing financial interests.

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