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Original Article

Prevalence of *Opisthorchis viverrini* and Its Associated Risk Factors in the Phon Sawan District of Nakhon Phanom Province, Thailand

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Abstract

Background: *Opisthorchis viverrini* is one of the endemic helminths in Thailand. Nonetheless, *O. viverrini* still exist and raise concerns regarding public health. This study aimed to evaluate the current prevalence of *O. viverrini* infections and underlying risk factors among rural communities in Northeast Thailand.

Methods: A cross-sectional study was conducted between Sep and Dec 2019 in Phon Sawan district in Nakhon Phanom Province, Thailand. The participants were selected using a voluntary sampling method after the proportional allocation of the total sample size. Demographic data were collected using a standardized questionnaire. One stool sample was collected from each participant and the presence of *O. viverrini* and other intestinal parasite infections were determined using the modified Kato-Katz technique.

Results: Overall, 250 participants aged 21 yr or older were enrolled. Overall, the prevalence of *O. viverrini* and *Strongyloides stercoralis* was 24% (95% confidence interval [CI]: 18.8-29.8) and 1.2% (95% CI: 0.2-3.5), respectively. Of the positive *O. viverrini* cases, the parasite rate significantly differed between gender, age group and in those who had a history of eating raw fish and taking the anti-helminthic drug (all $P < 0.05$). Nevertheless, multivariate regression analysis among *O. viverrini* cases revealed that only being male (adjusted odds ratio [aOR] 1.9 [95% CI: 1.1 – 3.6], $P = 0.033$) and aged ≥ 61 yr (aOR 6.7 [95% CI: 1.4 – 32.5], $P = 0.018$) were positively associated with a higher risk of *O. viverrini* infection.

Conclusion: Opisthorchiasis and strongyloidiasis are still endemic in this area and there is a need for projects to eliminate these parasites.



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Introduction

The Asian liver fluke, *Opisthorchis viverrini*, is a food-borne trematode parasite acquired by eating undercooked cyprinid fish containing infectious metacercaria. Opisthorchiasis remains a major public health problem in many countries particularly in the Lower Mekong Basin of Southeast Asia (1). In Thailand, over 8 million people are infected with *O. viverrini* (2), with the northeastern region recording a higher prevalence when compared with other regions in the country (3). *O. viverrini*, classified as a Class 1 carcinogen by The International Agency for Research on Cancer (IARC) in 2009, is commonly associated with the incidence of hepatobiliary diseases and the causative agents of bile duct cancer (cholangiocarcinoma) (4-6). Infections by these intestinal helminths are rarely diagnosed at an early stage of exposure due to its asymptomatic nature, exasperating the prevalence of opisthorchiasis in these regions (7).

A continuous effort has been undertaken by the Ministry of Public Health to eradicate the persistent *O. viverrini* infection among the rural population in Thailand, such as the Eco-Health/One Health approach (8) and the Cholangiocarcinoma Screening and Care Programme (9). These programs 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 northeastern regions, being among the highest affected areas in the world (2).

We aimed to describe epidemiological features of *O. viverrini* infection in a highly endemic district 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 Phon Sawan district.

Materials and Methods

Ethical approval

This study was approved by the Ethics Review Committee of the Nakhon Phanom Provincial Public Health Office (reference no. HE620026). 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 Sep and Dec 2019 in Phon Sawan district (latitude 17°27'23"N; longitude 104°28'7"E), Nakhon Phanom Province, Northeast Thailand (Fig.1). The district is predominantly rural and most residents live in villages as agriculturists growing rice and rubber. It covers an area of 719 km² and has a population of 43,860 people (Census 2010, Department of Provincial Administration, Thailand). The sample size for study participants was calculated using the following 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 17% from a previous study (2) and (e) is the allowed error margin which is set to 5%. In addition, contingencies were adjusted by adding another 10% of individuals, giving us a minimum of 239 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 standardized questionnaires in the 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, as well as present and type of domestic animal) and history of behavioral

aspects related to helminth infection (i.e. raw fish consumption and anti-helminthic drug

intake). All completed questionnaires were checked for accuracy and completeness.



Fig. 1: Map of Phon Sawan district (red) in Nakhon Phanom Province (beige) of northeastern Thailand where the study was conducted. Inset shows the map of Thailand

Sample collection and parasitological data

Study participants were invited to provide a single stool sample. Inclusion criteria were individuals who willing to participate in the study, aged ≥ 21 yr old and living in the area for at least six months. After proper instruction, a biohazard zipper bag containing a 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 the collection of the samples. All collected samples were checked for correct labeling and quantity of sample and transported immediately in a cool box to the field laboratory.

Modified thick smears (10, 11) were prepared after the stool collection using a commercially available Kato-Katz Kit (Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thai-

land). In this study, Kato-Katz thick smears were allowed to clear for 30 min before initially screened with a 10x objective under a light microscope. Suspected intestinal helminth objects were subsequently examined under a high-power objective. To eliminate bias, each fecal 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. Participants who were positive with helminths were treated with anti-helminthic drugs such as praziquantel and/or albendazole.

Statistical analysis

Survey data were double entered into a Microsoft Excel spreadsheet and cross-checked for errors. Data were processed and analyzed using Stata/SE version 13.1 for Windows (StataCorp, TX, USA). Differences in propor-

tions were tested using the Chi-squared test or 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 $P < 0.1$ from a likelihood ratio test in univariate analyses were entered into a multivariate logistic regression model and stepwise backward elimination was used to identify the main risk factors for infection. A $P < 0.05$ was considered statistically significant.

Results

Table 1: Prevalence of intestinal helminth infections among the population (N=250) living in Phon Sawan district, Nakhon Phanom Province, Thailand

<i>Intestinal parasitic infection</i>	<i>n (%)</i>	<i>95% CI</i>
Overall infection	60 (24)	18.8 - 29.8
<i>O. viverrini</i>	57 (22.8)	17.7 - 28.5
<i>O. viverrini</i> + <i>S. stercoralis</i>	3 (1.2)	0.2 - 3.5

n: Number of positive samples; CI: Confidence interval

Parasite prevalence

Of the overall participants, 60 (24%; 95% CI: 18.8-29.8) were positive for intestinal helminth infections (Table 1). Species distribution included a majority of *O. viverrini* mono-infections (n=57) [22.8%; 95% CI: 17.7-28.9], followed by three cases of double co-infections of *O. viverrini*/*S. stercoralis* (1.2%; 95% CI: 0.2-3.5).

Opisthorchiasis occurrence and risk factors

The specific prevalence and associated risk factors of *O. viverrini* are summarized in Table 2. Briefly, the overall prevalence of *O. viverrini*

Characteristics of participants

Overall, 250 participants (representing approximately 0.6% of the population) were screened for intestinal helminth infections (Table 1 and 2). The age of participants ranged between 21 to 78 yr (mean \pm standard deviation [SD] age of 52.3 ± 10.1 yr). The majority of the participants were females (58%), married (89.6%), worked as a farmer (97.6%), attended primary education (66.8%), and had a monthly income of Thai Baht (THB) ≤ 3000 (59.2%). Concerning variables directly related to helminth infections, the majority of participants had no history of eating raw cyprinid fish such as *Anematichthys repasson*, *Hampala dispar* and *Henicorhynchus lineatus* (50.8%), undertaken anti-helminthic drug in the past (70.4%) and less often living with domestic animals (57.2%) i.e. dogs (94.4%).

was significantly higher in males ($P=0.011$) and ≥ 61 age group ($P=0.005$), than in their counterparts. Similarly, with regards to participants' history, the prevalence differed significantly among those who had a history of eating raw cyprinid fish ($P=0.038$) as well as the used anti-helminth drugs ($P=0.025$). In terms of risk factors, the final model in multivariate logistic regression showed that only being male (adjusted odds ratio [aOR] 1.9 [95% CI: 1.1-3.6], $P=0.033$) and aged ≥ 61 yr (aOR 6.7 [95% CI: 1.4-32.5], $P=0.018$) were significantly associated with higher odds of contracting an *O. viverrini* infection.

Table 2: Specific prevalence of *Opisthorchis viverrini* and analysis of risk factors for infection in Phon Sawan district, Nakhon Phanom Province, Thailand

Characteristic	N (%)	Prevalence, n (%)	P-value ^a	COR (95%CI)	P value	AOR ^b (95%CI)	P value
Gender							
Male	105 (42)	34 (32.4)	0.011*	2.2 (1.2 - 3.9)	0.009*	1.9 (1.1 - 3.6)	0.033*
Female	145 (58)	26 (17.9)		1		1	
Age group (yr)							
21 - 40	25(10)	2 (8)	0.005*	1		1	
41 - 60	174 (69.6)	38 (21.8)		3.2 (0.7 - 14.2)	0.124	3.2 (0.7 - 14.5)	0.132
≥61	51 (20.4)	20 (39.2)		7.4 (1.6 - 34.9)	0.011*	6.7 (1.4 - 32.5)	0.018*
Education level							
Primary	167 (66.8)	43 (25.8)	0.909	1.4 (0.7 - 2.9)	0.358	0.9 (0.4 - 2.1)	0.819
Secondary	56 (22.4)	11 (19.6)		1		1	
Tertiary	27 (10.8)	6 (22.2)		1.2 (0.4 - 3.6)	0.785	1.1 (0.3 - 3.4)	0.903
Marital status							
Single	26 (10.4)	6 (23.1)	0.999	1		1	
Married	224 (89.6)	54 (24.1)		1.1 (0.4 - 2.8)	0.907	1.2 (0.4 - 3.2)	0.748
Monthly income							
THB≤3000	148 (59.2)	38 (25.7)	0.547	1.3 (0.7 - 2.3)	0.455	1.1 (0.6 - 2.2)	0.697
THB>3001	102 (40.8)	22 (21.6)		1		1	
Occupation							
Farmer	244 (97.6)	59 (24.2)	0.670	1.6 (0.2 - 13.9)	0.673	1.4 (0.1 - 12.3)	0.796
Laborer	6 (2.4)	1 (16.7)		1		1	
History of eating raw cyprinid fish ^c							
Yes	123 (49.2)	23 (18.1)	0.038*	1		1	
No	127 (50.8)	37 (30.1)		0.5 (0.3 - 0.9)	0.028*	0.6 (0.3 - 1.1)	0.101
History of taking anti-helminthic ^d							
Yes	74 (29.6)	25 (33.8)	0.023*	2.1 (1.1 - 3.8)	0.020*	1.8 (0.9 - 3.5)	0.059
No	176 (70.4)	35 (19.9)		1		1	
Having domestic animal							
Yes	107 (42.8)	23 (21.5)	0.457	1		1	
No	143 (57.2)	37 (25.9)		1.3 (0.7 - 2.3)	0.423	1.6 (0.8 - 3.0)	0.160
Type of domestic animal							
Dog	101 (94.4)	21 (20.8)	0.468	1		1	
Cat	6 (5.6)	2 (33.3)		1.9 (0.3 - 11.1)	0.474	1.6 (0.3 - 10.7)	0.607

^aThe P values for differences in positivity rates between categories were calculated based on Fisher's exact test or Chi-square test.^bAdjusted for variables with a P<0.1 from a likelihood ratio test in univariate analysis.^cThe type of freshwater fishes including *A. repasson*, *H. dispar* and *H. lineatus*.^dThe type anti-helminthic drugs including praziquantel and/or albendazole

*Significant difference P<0.05. COR: Crude odd ratio; AOR: Adjusted odd ratio; CI: Confidence interval; THB: Thai Bhat

Discussion

The present study showed that the overall prevalence of *O. viverrini* was high (24%) and almost half of respondents (49.2%) also reported consuming raw fish in the Phon Sawan district. Our study setting is situated in the northeastern region of Thailand. The northeastern region consistently showed the highest incidence of opisthorchiasis as compared to other regions in the country (3, 10-12). 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 (13). 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 of *O. viverrini* infection.

Furthermore, 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*. A similar pattern of prominence favoring *O. viverrini* over *S. stercoralis* was also observed in several studies in Thailand. 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 (14-18), further highlighting the prevalence of these parasitic infections.

The male gender was a significant risk factor for OV infection is consistent with the results of another recent study in northeastern Thailand (6, 10, 11, 19) and is supported by an earlier study (20). While other surveys in Thailand have failed to show any statistically significant association between gender and infection (21, 22), all gender differences have indicated a higher prevalence in males. In the present study, the strength of the association with the male gender was high – the odds of males be-

ing infected were almost two times higher than those for females. The disparity in infection distribution among the genders may be attributed to behavioral factors and socialization patterns that have men consuming more raw cyprinid fish than females, especially among the rural communities in Thailand (21, 23). 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 (19); this is especially true among those involved in farming and labor-intensive occupation typically male-dominated.

The prevalence of *O. viverrini* infection in the present study was significantly different among age groups, particularly higher in those of >60 years. This is similar to previous findings where age-specific patterns have been observed for *O. viverrini* infection in other parts of Thailand (10, 11, 24, 25), Laos (26), and Cambodia (27). Although older adults have a significant difference in prevalence among the age groups in our study, people of all ages are at risk of being infected with *O. viverrini*. Moreover, morbidity due to chronic *Opisthorchis*-associated cancer (i.e. cholangiocarcinoma) can occur early in the course of the disease in infected individuals. This may present the greatest part of the disease burden associated with opisthorchiasis for the affected families and the entire communities (28, 29). The interrelation of *O. viverrini* infection and age becomes a serious public health issue and requires intergenerational and transgenerational approaches in designing health education campaigns, such as delivering tailored health messages and measures to each specific age group (30).

In general, the prevalence of *O. viverrini* and *S. stercoralis* infections in Thailand gradually increases in an age-dependent manner. In the northeastern region of Thailand, older participants (>60 yr) had the highest prevalence in both *O. viverrini* and *S. stercoralis* infection as

compared to the younger age groups (6). A similar age-specific pattern has been observed for *O. viverrini* infection in other parts of Thailand (10, 11, 18, 24). Other than having consumed a greater amount of raw fish, the fact that older participants had a high prevalence of *O. viverrini* may be also related to poverty (31). Furthermore, the reason for a high prevalence of strongyloidiasis in older participants may be due to them having more prolonged exposure to sources of *S. stercoralis* infection (32). The majority of the older population in this region work in agriculture, thus they have greater contact with soil than those in other age groups.

Several limitations should be considered in this study. First, while the convenience sampling approach used in this study was efficient and cost-effective, it is more likely to be flawed by selection bias. The survey was conducted in a rural community, meaning that residents working in agriculture were disproportionately represented. Second, the survey underreported children in the population have led to an underestimation of overall data. Although liver fluke infections are largely associated with adults due to the behavior of consuming undercooked fish, the opisthorchiasis status in children is not well characterized and warrant further investigation. Third, due to the imperfect sensitivity of the Kato-Katz technique, the true prevalences of *O. viverrini* and *S. stercoralis* may be much higher than the prevalence reported in this study.

Conclusion

Despite the intensive national parasite control program, *O. viverrini* and *S. stercoralis* are persisted in rural areas of northeast Thailand. The findings in the present study would provide critical data that can be used towards improving current measures in eradicating the disease. Awareness campaigns and appropriate control programs should be developed to reduce intestinal parasitic infection, especially in

agriculturists in rural communities. Additionally, these results should encourage policymakers and public health personnel to improve programs for parasitic control and health promotion.

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Conflict of interest

The authors declare that they have no competing interest.

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