sciendo ASIAN BIOMEDICINE DOI 10.1515/abm-2018-0030 — Asian Biomed (Res Rev News) 2018; 12(1):45–51

Brief communication (original) **Open access**

Risk factors for *Opisthorchis viverrini* infection in Nakhon Phanom, Thailand, where the infection is highly endemic

Songkran Nakbun, Pramote Thongkrajai, Choosak Nithikathkul*

Abstract

Background: Opisthorchiasis caused by *Opisthorchis viverrini* is a serious health issue in the Mekong basin region, resulting in a high prevalence of cholangiocarcinoma. Nakhon Phanom province had the highest prevalence of *O. viverrini* infection in Thailand at 60% of the surveyed population in 2009, despite the attempted control of opisthorchiasis for >50 years. Knowing risk factors for *O. viverrini* infection in Nakhon Phanom may lead to improved control and prevention of opisthorchiasis.

Objectives: To determine risk factors for *O. viverrini* infection in Nakhon Phanom.

Methods: We conducted a cross-sectional survey in Nakhon Phanom province from February to March 2014. The community was selected using a stratified random sampling method, and then, participants were selected by systematic random sampling. Individuals ≥15 years old were included. Knowledge of *O. viverrini* infection, and attitudes and practice to avoid it were assessed using a questionnaire. *O. viverrini* infection was determined by stool examination with a formalin–ether concentration method. Factors associated with the infection were determined using multivariate logistic regression analysis.

Results: Of the 134 participants, 75 (56%) were infected with *O. viverrini*. In the multivariate logistic regression analysis, 3 independent factors were associated with *O. viverrini* infection: age ≥ 55 years, odds ratio (OR) adjusted 6.36 (95% confidence interval (CI) 1.28–31.66); consumption of chopped raw-fish salad (*koi pla*), OR adjusted 28.74 (95% CI 3.59–230.24); and perceived susceptibility, OR adjusted 0.15 (95% CI 0.03–0.74).

Conclusions: Age ≥ 55 years, consuming *koi pla*, and perceived susceptibility were independently associated with *O. viverrini* infection in Nakhon Phanom.

Keywords: endemic diseases, opisthorchiasis, *Opisthorchis viverrini*, risk factors, trematode infections

Opisthorchiasis, caused by the liver fluke *Opisthorchis viverrini*, is a major risk factor for cholangiocarcinoma [1]. Cholangiocarcinoma is a malignant cancer of the biliary epithelium arising from within either the intrahepatic or extrahepatic bile ducts, with an extremely poor prognosis in humans [2]. Opisthorchiasis is foodborne trematode infection

***Correspondence to: Choosak Nithikathkul,** Tropical and Parasitic Diseases Research Unit, Graduate Studies Division, Faculty of Medicine, Mahasarakham University, Mahasarakham 44000, Thailand, e-mail: choosak.l@msu.ac.th

Tropical and Parasitic Diseases Research Unit, Graduates Studies Division, Faculty of Medicine, Mahasarakham University, Mahasarakham 44000, Thailand

and is prevalent where raw cyprinid fish, or freshwater carp, are a staple of the diet. Trematodes in the form of cercariae, or the parasitic flukes or worms, that form part of the life cycle of *O. viverrini* are released from freshwater snails of the genus *Bithynia*, and attach to and penetrate the skin of susceptible cyprinid species to form an infective stage, the metacercaria. When infected fish are consumed raw, the adult metacercaria of *O. viverrini* inhabit the biliary system. The embryonated eggs of gravid flukes containing a miracidium are discharged with the bile and eventually passed into the environment in the fecal stream. After reaching freshwater, the eggs are ingested by *Bithynia*. These snails are abundant in reservoirs with shallow water, rice fields, and wetlands, and are frequently found in water bodies close to villages where high fecal contamination occurs. Within the snail, eggs hatch to release miracidia, which transform to sporocysts. Then sporocysts undergo asexual reproduction to give rise to rediae and finally cercariae that infect the freshwater carp, and so, the cycle continues [3].

Despite the attempted control of opisthorchiasis for >50 years [1, 3, 4], opisthorchiasis remains a serious health issue in the Mekong basin, particularly in the northeast of Thailand, resulting in a high prevalence of cholangiocarcinoma in this region [3, 5, 6]. The prevalence of *O. viverrini* infection found by nationwide surveys is consistently high in the northeast of Thailand from 15.5% in 2000 to 16.6% in 2009 [3]. Nakhon Phanom province, in northeast Thailand and close to the Mekong river, had the highest prevalence of *O. viverrini* infection in Thailand at 60% of the surveyed population in 2009 [3]. The prevalence of *O. viverrini* infection in Nakhon Phanom remains highest among 7 provinces in the upper northeastern region of Thailand at 40.9%. Nevertheless, Sakon Nakhon province had a high prevalence of 32.1%, followed by Nong Khai (24.3%), Nong Bua Lam Phu (23.6%), Udon Thani (19.5%), Loei (17.5%), and Bueng Kan (17%) [7]. However, this prevalence is probably grossly underestimated as suggested by estimates using newer polymerase chain reaction-based diagnostic techniques [8].

Nationwide studies of factors associated with *O. viverrini* infection in each area of Thailand found that sex and education were significant factors in *O. viverrini* infection in an urban area of Mahasarakham province where overall prevalence was 15% [9]. Sex, age, and eating raw or fermented fish were significantly associated with *O. viverrini* in the northeast of Thailand in a large population in which the overall prevalence of opisthorchiasis was 22.7% [7], eating raw fish and age were significant factors in area *O. viverrini* infection in a rural area of Chacheongsao province where the prevalence was 26.2% [10]. Use of anthelmintic in the past, eating raw fish, and the unsafe disposal of waste food were significant factors in Nong Khai province where the prevalence of *O. viverrini* infection was 33% [11]. Previous use of praziquantel and lack of knowledge about anthelmintics were significant factors in Yasothon province, Northeast Thailand, where the prevalence of *O. viverrini* infection was 37.2% [6]. All these studies concluded that the individual habit of eating raw or undercooked fish is the primarily associated with the liver fluke infection. However, other factors associated with *O. viverrini* infection were both similar and different in each area where there are different prevalence of *O. viverrini* infection.

The high prevalence areas were associated with the consumption of raw or undercooked fish [12], especially in Nakhon Phanom because of the strong livelihood and lifestyle relationships in the wetlands ecosystem, which cannot be separated from eating habits [12]. Attitude is an important factor that is associated the consumption of raw fish and *O. viverrini* infection. More understanding is needed of the attitudes and practices associated with the practice of eating raw fish [12]. Previous studies have almost entirely focused of the practice of consuming raw or undercooked fish.

It is crucial to identify knowledge, attitudes, and practice (KAP) to discriminate and identify the constructs that may pose barriers to controlling infectious diseases [13]. The health belief model (HBM) focuses on belief and attitude [14], and is important to discriminate the categories and indicators of the risk factors for opisthorchiasis.

The present study investigated risk factors for *O. viverrini* infection that are associated with knowledge, attitude (HBM), and practice or behavioral data from Nakhon Phanom, Thailand, an area of the Mekong basin region where opisthorchiasis is highly endemic. Knowing the important risk factors for *O. viverrini* infection in this particular area may contribute to improve control and prevention of opisthorchiasis.

Methods

After approval of the study protocol by the Mahasarakham University Ethics Committee for Human Research (reference number 0091/2556), we conducted a cross-sectional survey in a community in Nakhon Phanom province, Thailand, from February to March 2014. The community was selected by stratification using a random sampling method by sampling from the district and subdistrict levels to the community level. The inclusion criteria were individuals who were ≥15 years old and had lived in the province ≥6 months. A list of individuals was selected from health care centers using the inclusion criteria, and then, the participants were selected by systematic random sampling.

The required sample size of 134 people was calculated using the following formula:

$$(n) = \frac{Z^2_{\alpha/2} NP(1-P)}{Z^2_{\alpha/2} P(1-P) + (N-1)e^2}$$

where,
n = sample size,
N = population = 201,
Z = 1.96 95% ($\alpha = 0.05$),
e = acceptable margin of error = 0.05,
and P = prevalence of *O. viverrini* infection = 0.6 [3].

The rural community studied is located near the Mekong River, and most of the communities are employed in agriculture. After participants provided written informed consent (when participants were <18 years old, their written informed assent with written informed consent was provided by a parent or legal guardian) on a form approved by the ethics committee, all eligible participants were asked to complete a questionnaire to obtain demographic information, and assess KAP regarding *O. viverrini* infection. Trained research assistants collected data by interview. Stool samples were also collected and examined for *O. viverrini* using a modified formalin–ether concentration technique (FECT) [15].

Knowledge regarding *O. viverrini* infection was assessed using a questionnaire with 14 items. A correct answer scored 1 and an incorrect answer 0, and the test score was categorized as mild (if the test score was <9) and good (if the test score was 9–14). Attitude in the HBM, such as susceptibility, severity, benefits, and barriers, was scored. Each term comprised 7 items. A response score was categorized on a Likert-type scale of strongly agree (positive question scored 5, negative question scored 1), agree (positive question scored 4, negative question scored 2), neutral (positive and negative question scored 3), disagree (positive question scored 2, negative question scored 4), and strongly disagree (positive question scored 1, negative question scored 5). Each term was categorized as mild (if the score was <24) and good (if the score was 24–35). The practice component was divided into 3 parts. Part 1 comprised individual eating raw-fish habit, which included 5 items. Each item was categorized into 2 levels, being not eat and eat. Part 2 comprised family and friends eating raw-fish habit, which included 2 items. Family eating raw-fish habit item was categorized into 2 levels, being someone in the family and nobody in the family with habit of eating raw fish. Friends eating raw-fish habit item were categorized into 2 levels, being no friends and some friends with a habit of eating raw fish. Part 3 comprised community eating raw-fish habit. The item was categorized into 2 levels, being nobody and somebody in community with a habit of eating raw fish.

The participants were categorized into groups of those infected with *O. viverrini* or not infected, based on evidence of *O. viverrini* infection from the stool examination. The factors were compared between groups by unconditional logistic regression analysis to determine the significant factors associated with *O. viverrini* infection. First, univariate analysis was conducted. Variables significant at the $P < 0.25$ level were included in a multivariate analysis using binary logistic regression analysis with backward elimination. In the final model, significance was set at $P < 0.05$.

All statistical analyses were performed using SPSS for Windows (version 16.0; SPSS Inc.).

Results

There were 134 participants, and 75 (56%) were infected by *O. viverrini*. Baseline characteristics are shown in **Table 1**.

There were 4 significant factors in univariate logistic regression in terms of KAP associated with *O. viverrini* infection, including age (≥55 years; odds ratio (OR) crude 5.11, 95% CI 1.23–21.28), perceived susceptibility (OR crude

Table 1. Baseline characteristics of participants

Characteristics	Number (N = 134)	%
Sex		
Male	52	39
Female	82	61
Age, years	n (mean age, SD)	
15–24	12 (18.08, 3.66)	9
25–34	13 (30.92, 2.43)	10
35–44	46 (39.74, 2.70)	34
45–54	31 (49.94, 2.74)	23
55+	32 (61.25, 5.29)	24
Mean (SD) years	44.4 (13.25)	
Marital status		
Single	12	9
Married	107	80
Divorced/widowed	15	11
Education		
Elementary	103	77
Junior high school	8	6
High school	17	13
Higher education	6	4
Occupation		
Housewife	11	8
Farmer	77	57
Laborer	24	18
Other	22	17

SD, standard deviation

0.14, 95% CI 0.03–0.64), consumption of chopped raw-fish salad (*koi pla*) (OR crude 29.95, 95% CI 3.85–233.12), and consumption of briefly fermented fish stuffed with rice (*pla jom*) (OR crude 2.36, 95% CI 1.00–5.52) (**Table 2**). After multivariate analysis, there were 3 independent factors remaining that were associated with *O. viverrini* infection in the final model: age (≥55 years; OR adjusted 6.36, 95% CI 1.28–31.66), perceived susceptibility (OR adjusted

Table 2. Personal and community information regarding knowledge, attitude, and practice associated with *Opisthorchis viverrini* infection: univariate analysis

Factors	Noninfected (n = 59)	%	Infected (n = 75)	%	*P*	OR crude	95% CI	
							Lower	Upper
Sex								
Male	21	36	31	41	–	1	–	–
Female	38	64	44	59	0.50	0.78	0.39	1.59
Age, years								
15–24	8	14	4	5	–	1	–	–
25–34	8	14	5	7	0.79	1.25	0.24	6.44
35–44	21	35	25	33	0.20	2.38	0.63	9.03
45–54	13	22	18	24	0.15	2.77	0.69	11.19
55+	9	15	23	31	0.03*	5.11	1.23	21.28
Marital status								
Single	8	14	4	5	–	1	–	–
Married	46	78	61	81	0.13	2.65	0.75	9.35
Divorced/widowed	5	8	10	14	0.10	4.00	0.80	20.02
Education								
Elementary	42	71	61	81	–	1	–	–
Junior high school	3	5	5	7	0.08	7.26	0.82	64.42
High school	9	15	8	11	0.11	8.33	0.63	110.02
Higher education	5	9	1	1	0.21	4.44	0.42	46.55
Occupation								
Housewife	7	12	4	5	–	1	–	–
Farmer	30	51	47	63	0.13	2.74	0.74	10.17
Laborer	9	15	15	20	0.16	2.92	0.66	12.82
Other	13	22	9	12	0.80	1.21	0.27	5.40
Knowledge								
Mild	43	73	63	84	–	1	–	–
Good	16	27	12	16	0.12	0.51	0.22	1.19
Perceived susceptibility								
Mild	2	3	15	20	–	1	–	–
Good	57	97	60	80	0.01*	0.14	0.03	0.64
Perceived severity								
Mild	3	5	8	11	–	1	–	–
Good	56	95	67	89	0.25	0.45	0.11	1.77
Perceived benefits								
Mild	11	19	6	8	–	1	–	–
Good	48	81	69	92	0.07	2.64	0.91	7.61
Perceived barriers								
Mild	39	66	51	68	–	1	–	–
Good	20	34	24	32	0.82	0.92	0.44	1.90
Chopped raw-fish salad (*koi pla*)								
Not eat	17	29	1	1	–	1	–	–
Eat	42	71	41	99	<0.01*	29.95	3.85	233.12

(*Continued*)

Table 2. Personal and community information regarding knowledge, attitude, and practice associated with *Opisthorchis viverrini* infection: univariate analysis (*Continued*)

Factors	Noninfected (n = 59)	%	Infected (n = 75)	%	*P*	OR crude	95% CI	
							Lower	Upper
Briefly fermented fish (*pla som*)								
Not eat	14	24	9	12	–	1	–	–
Eat	45	76	66	88	0.08	2.28	0.91	5.72
Briefly fermented fish stuffed with rice (*pla jom*)								
Not eat	17	29	11	15	–	1	–	–
Eat	42	71	64	85	0.049*	2.36	1.00	5.52
Sawn raw fermented fish (*jaewbhong*)								
Not eat	3	5	3	4	–	1	–	–
Eat	56	95	72	9	0.76	1.29	0.25	6.62
Raw fermented fish (*pla ra*)								
Not eat	1	2	4	5	–	1	–	–
Eat	58	98	71	95	0.30	0.31	0.03	2.81
The family had consumption of raw fresh fish								
No	7	12	19	25	–	1	–	–
Yes	52	88	56	75	0.06	0.40	0.15	1.02
Have a friend who consumes raw fresh fish								
No	7	12	6	8	–	1	–	–
Yes	52	88	69	92	0.46	1.55	0.49	4.88
The people in community had consumed raw fresh fish								
No	14	24	24	32	–	1	–	–
Yes	45	76	51	68	0.29	0.66	0.31	1.43

*$P < 0.05$
OR, odds ratio; CI, confidence interval

0.15, 95% CI 0.03–0.74), and consuming chopped raw-fish salad (*koi pla*) (OR adjusted 28.74, 95% CI 3.59–230.24) (**Table 3**). The participants in the 55+ years age group had about 6.36 times higher risk of opisthorchiasis than those in the 15–24-year age group. Participants with good perceived susceptibility had *decreased* risk factors for opisthorchiasis of 85% compared with the participants with poor perceived susceptibility. For participants consuming *koi pla*, the risk of opisthorchiasis was >28.74 times than that of participants who do not consume *koi pla*.

Discussion

The present study was conducted in a highly endemic area of *O. viverrini* infection and found that consuming *koi pla* is the main risk factor for *O. viverrini* infection in Nakhon Phanom. Participants who consumed *koi pla* were 29 times more likely to have *O. viverrini* infection (**Table 3**). Not only as reported here in the highly endemic area, *koi pla* consumption was also previously reported as a high-risk factor for infection at the national level [7, 10, 11]. *Koi pla* is an unfermented raw-fish dish that is a popular traditional component of the diet in the northeastern region of Thailand. Unlike in fermented fish dishes such as *pla ra*, viable metacercariae may be found resulting in a high infection rate [5]. The fish in the Mekong basin are a vital source of fish protein and nutrients in this region. According to the tradition of rural areas of the Mekong basin, *koi pla* is popular because of its apparent good taste and short preparation time [1, 3, 12]. *Koi pla* is easily made from unfermented raw fish. Food sharing culture is still prevalent among relatives and in the community [16]. The present report supports consuming *koi pla* as a risk factor for opisthorchiasis and is consistent with previous reports [10].

The local and deep culture surrounding the consumption of raw fish makes it staple favorite. The elderly passionately consume raw fish and are more the familiar with the taste of *koi pla* than adolescent and young children [12]. The participants in the 55+ years age group have a risk factor for opisthorchiasis about 6.36 times of those in the group aged 15–24 years (**Table 3**), as consistent with previous reports of studies conducted in Nakhon Phanom and Baan Nayao village in a rural area of Chachoengsao [7, 10]. The present

Table 3. Factors associated with *Opisthorchis viverrini* infection: multivariate analysis

Factors	Noninfected (n = 59)	%	Infected (n = 75)	%	*P*	OR adjusted	95% CI	
							Lower	Upper
Age, years								
15–24	8	14	4	5	–	1	–	–
25–34	8	14	5	7	0.97	1.04	0.17	6.25
35–44	21	35	25	33	0.07	4.10	0.89	19.03
45–54	13	22	18	24	0.15	3.21	0.66	15.64
55+	9	15	23	31	0.02*	6.36	1.28	31.66
Perceived susceptibility								
Mild	2	3	15	20	–	1	–	–
Good	57	97	60	80	0.02*	0.15	0.03	0.74
Chopped raw-fish salad (*koi pla*)								
Not eat	17	29	1	1	–	1	–	–
Eat	42	71	74	99	<0.01*	28.74	3.59	230.24

*$P < 0.05$

OR, odds ratio; CI, confidence interval

study adds that participant perception of increased risk of the *O. viverrini* infection reduces the risk of opisthorchiasis. The *O. viverrini* infection may be reduced by 85% if the participants had perceived susceptibility (**Table 3**). HBM studies indicate that disease perception is related to disease prevention by changing eating behavior [17]. By contrast, if the participants were not aware or had no perceived susceptibility to *O. viverrini* infection, they may still eat *koi pla* and take anthelmintic drugs afterward [17]. These behaviors may be related to eating habit, which is maintained in part because of their fondness for the taste of raw fish. Taking praziquantel may actually increase the risk of cholangiocarcinoma in *O. viverrini*-infected patients [17, 18]. To reduce *O. viverrini* infection rate in highly endemic areas, further intervention regarding perception of *O. viverrini* infection is encouraged, especially to increase perceived susceptibility. Perceived susceptibility can be encouraged, but this is most often focused on creating a sense of fear or awareness of being threatened [14]. This limitation can be reduced by applying the perceived susceptibility with self-efficacy and perceived control. Strengthening self-efficacy often focuses on strengthening self-confidence in what needs to be done and results in action or behavioral change [14].

A major strength of the present study is that it was conducted in an area highly endemic for *O. viverrini* infection. Limitations include that data were gathered by using the study questionnaire, which may not be entirely accurate. Some participants may not respond truly or fail to disclose or hide some information. Eating behaviors were not observed directly. The prevalence of infection may be underestimated by the FECT.

Conclusions

Being in the >55-year age group, consuming *koi pla*, and perceived susceptibility were independently associated with *O. viverrini* infection in Nakhon Phanom, an area in northeast Thailand that is highly endemic for the infection. The HBM construct of perceived susceptibility is an important contribution to knowledge to reduce the prevalence of opisthorchiasis in highly endemic areas. Further intervention regarding perception of *O. viverrini* infection and efforts to change behavior surrounding *koi pla* consumption are encouraged.

Author contributions. All authors contributed substantially to the conception and design of the study. SN acquired, analyzed, and interpreted the data. SN and CN drafted the manuscript. All authors critically revised the article, approved the final version submitted for publication, and take responsibility for the statements made in the published article.

Acknowledgments. This research was supported financially by Research Promotion Division at the Mahasarakham University, the Faculty of Medicine Mahasarakham University, and a Bhumibol scholarship. We thank the Boromarjonani College of Nursing, Nakhon Phanom University, and the Faculty of Medicine, Mahasarakham University, for their encouragement.

Conflict of interest statement. The authors have each completed and submitted an International Committee of Medical Journal Editors Uniform Disclosure form for potential conflicts of interest. None of the authors has anything to disclose.

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