RESEARCH ARTICLE

Risk factors for *Opisthorchis viverrini* Infection in Nong Khai Province, Thailand

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Abstract

Background: *Opisthorchis viverrini* (OV) infection is the main risk factor for cholangiocarcinoma and is often found in Northeastern Thailand. The prevalence of OV infection and the incidence of cholangiocarcinoma are major public health problems in this region. Objectives: The objectives of this study were to identify factors associated with OV infection among people in Nong Khai Province in order to develop a prevention programme in the community. Materials and Methods: The data were collected in July 2013. Stool specimens were examined for intestinal parasites within hours after collection using a normal saline wet preparation and the modified Kato-Katz technique. A case-control study was conducted to collect information about demographic data, the habit of eating unsafely prepared fish, the safe disposal of waste food, and the practice of defaecating in fields. Structured questionnaires were used to interview 351 participants (117 cases and 234 controls) in a random selection of 30 villages across Nong Khai Province. Multiple logistic regression was used to identify risk factors for OV infection. Results: In the multivariate analysis, the results showed that the factors which had a statistically significant association with OV infection were the habit of consuming unsafely prepared fish (OR_{adj}=5.17, 95%CI=2.49-10.74), the similar habit of family members (OR_{adj}=3.25, 95%CI=1.63-6.49) , a history of O. viverrini infection (OR_{adj}=5.64, 95%CI=2.10-15.18), a history of taking praziquantel (OR_{adj}=5.66, 95%CI=3.11-10.29), and the unsafe disposal of waste food (OR_{adj}=2.1, 95%CI=1.10-3.80). Conclusions: The findings of this study highlight the features on which a community programme should focus in order to reduce the prevalence of opisthorchiasis and incidence of bile duct cancer.

Keywords: ***Opisthorchis viverrini*** - risk factor - Nong Khai, Thailand

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Introduction

Opisthorchis viverrini (OV) is a significant risk factor for cholangiocarcinoma (Sripa et al., 2012), and people become infected with OV when they eat raw, undercooked or under-fermented cyprinoid fish which contain an intermediate form of the parasite. OV infection is endemic in the Lower Mekong Basin where there is a strong cultural tradition of eating improperly prepared freshwater fish (Sithithaworn et al., 2012).

While high prevalences of O. viverrini infection are commonly found in the countries of Southeast Asia, infection rates in Thailand have been reported to be the highest in the world with an estimated six million Thai people infected, and the highest rates within Thailand are in the northeast region where provincial incidence rates have varied between 4.6% and 60.8% (Sithithaworn et al., 2012). Large variations in infection rates by intestinal parasites (mainly OV) can also occur within a province (Songserm et al, 2012).

The aim of this study was to identify factors associated with OV infection among people in Nong Khai Province, which is located along the Mekong River, as the basis for developing a prevention programme in the local communities. Nong Khai has one of the highest rates of OV infection in the upper region of Northeast Thailand (Thaewnongiew et al., 2014). Although much is already known about the risk factors for OV infection, these needed to be confirmed for Nong Khai.

Materials and Methods

This was a population-based case-control study conducted in Nong Khai Province, which is in the northeast region of Thailand, and is situated along a part of the Mekong River, which forms the border between Thailand and Lao PDR. The province covers an area of 3,027.3 km^2 and is divided into nine districts which include 705 villages. The total population in 2011 was 509, 870. The province is predominantly low-lying and rural with agriculture being the main source of employment, and fish plays an important part in the diet of local communities, especially those alongside the Mekong River.

Survey

A survey of OV infection in Nong Khai Province was

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carried out during 1-10 July 2013. The subjects were selected by multistage sampling with a cluster size of 30. The estimated sample size required was 516 and was calculated using the following formula:

$$\frac{Z^2_{(\alpha/2)}NP_{(1-p)} \text{ x design effect}}{Z^2_{(\alpha/2)}P_{(1-p)} + (N-1)d^2}$$

N=population=509,870
Z=1.96 95% (∞= .05)
design effect: df=2
d=acceptable margin of error=0.05
P=estimated prevalence rate OV infection=0.14 (datum supplied by Office of Disease and Control 6)

Simple random sampling was used to select 30 villages and also the selection of 30 households from the household register for each village. One subject over 15 years of age, who had been living in the village for at least six months, was randomly chosen within each household at the time of visiting by a research assistant. The visits were usually made at the end of the day, and if no-one was home, a neighbouring household was selected. A total of 900 eligible subjects (30 villages x 30 households in each village) were invited to participate, and 526 (58.4%) provided informed consent and a stool sample, which was analysed within hours after collection using a normal saline wet preparation and the modified Kato-Katz technique. Information about demographic characteristics, and the practice of eating unsafely prepared fish was collected on interview by trained research assistants. Unsafely prepared fish was defined as raw, undercooked or improperly fermented freshwater fish. Information was also collected about the household's usual way of disposing of waste food. Disposal by giving it to dogs or cats or discarding it under the house or in open pits to which animals had access was regarded as unsafe. The

Table 1. Association of Demographic and Other Factors with *Opisthorchis viverrini (OV)* Infection: Univariate Analysis

Variables	Cases (n=117) Number (%)	Controls (n=234) Number (%)	ORcrude	95%CI	p-value
Age (years)					
15-34	19 (16.2)	89 (38.0)	1	-	-
35-54	60 (51.3)	71 (30.3)	3.96	2.17-7.23	<0.001
55+	38 (32.5)	74 (31.6)	2.41	1.28-4.52	<0.001
Gender					
Female	51 (43.6)	132 (56.4)	1	-	-
Male	66(56.4)	102 (43.6)	1.67	1.04-2.69	0.015
Education					
Primary school/lower	84 (71.8)	113 (48.3)	1	-	-
Secondary school/upper	33 (28.2)	121 (51.7)	0.37	0.22-0.61	<0.001
Occupation					
Agriculture	107 (91.5)	198 (84.6)	1	-	-
Others	10 (8.5)	36 (15.4)	0.51	0.23-1.11	0.049
History of O. viverrini infection					
Never	90 (76.9)	225 (96.2)	1	-	-
Ever	27 (23.1)	9 (3.8)	7.5	3.25-18.75	<0.001
History of taking praziquantel					
Never	47 (40.2)	193 (82.5)	1	-	
Ever	70 (59.8)	41 (17.5)	7.01	4.13-11.93	<0.001
Family income					
Not enough	25 (21.4)	49 (20.9)	1	-	-
Enough	92 (78.6)	185 (79.1)	0.97	0.55-1.76	0.515
Habit of eating unsafely prepared fish					
Not eat	15 (12.8)	128 (54.7)	1	-	-
Eat	102(87.2)	106 (45.3)	8.21	4.40-16.04	<0.001
Habit of eating unsafely prepared fish by family members					
Not eat	24 (20.5)	126 (53.8)	1	-	-
Eat	93 (79.5)	108) (46.2)	4.52	2.63-7.92	<0.001
Habit of eating unsafely prepared fish by close friends					
Not eat	44 (37.6)	144 (61.5)	1	-	-
Eat	73 (62.4	90) (38.5)	2.22	1.36-3.62	<0.001
Habit of eating unsafely prepared fish by neighbours					
Not eat	36 (30.8)	134 (57.3)	1	-	-
Eat	81 (69.2)	100 (42.7)	2.61	1.54-4.52	<0.001
Safe disposal of waste food					
Yes	34 (29.1(83)	40.0 (1)	-	-	
No	83 (70.9)	151) (60.0)	1.34	0.81-2.24	0.139
Defaecation in fields (no latrine)					
Yes	41 (35.0)	67 (28.6)	1	-	-
No	76 (65.0)	67 (71.4)	0.74	0.45-1.23	0.135

habit of defaecating in fields was also explored.

Cases and controls

The cases were the 117 participants who were found to have a positive test result for OV infection. Two controls were selected for each case, and 234 controls were randomly chosen from the 409 participants with negative results. All the 117 participants with a positive test result were treated with praziquantel.

Statistical analysis and ethical approval

The associations between case-control status and potential risk factors were analysed using unconditional logistic regression. Variables significant at the p<0.25 level in a univariate analysis were included in a multivariate analysis with backward elimination. Additional candidate variables were factors which were non-significant in the univariate analysis, but which have been reported as strong risk factors for OV infection in previous studies. Statistical analyses were performed using Stata version 10.0 (StataCorp LP, 2007). Statistical significance in the final model was set at p≤0.05.

The research was approved by the Khon Kaen University Ethics Committee for Human Research (reference no. HE562259).

Results

The demographic characteristics of the participants are summarized in Table 1. Just over half (52.1%) of the total number of participants (cases + controls) were female, and the participants were approximately equally distributed between the three age groups. Most (56.1%) had not progressed beyond primary school, the vast majority (89.9%) was employed in agriculture, and 78.9% reported that their family income was sufficient.

Table 1 also shows the results of the univariate analysis. Factors associated with the status of OV positive were age, gender, education, occupation, a history of OV infection, a history of taking praziquantel, the habit of eating unsafely prepared fish, and the same habits of family members family members, close friends and neighbours (P-value<0.05).

Table 2 shows the results of the final model of the multivariate analysis. Factors remaining associated with the status of a positive OV infection were a history of OV infection (ORadj=5.64, 95%CI=2.10-15.18), a history of taking praziquantel (ORadj=5.66, (95%CI=3.11-10.29), a habit of eating unsafely prepared fish (ORadj=5.17, 95%CI=2.49-10.74), the same habit of family members (ORadj=3.25, 95%CI=1.63-6.49), and the unsafe disposal of waste food (ORadj=2.4, 95%CI=1.10-3.80).

Discussion

The findings of this study confirmed the association between a history of OV infection and previous treatment with praziquantel and current infection. These findings are in line with the study by Saengsawang et al. (2013) who found that the odds of infection of those who had taken praziquantel were 2.31 times higher than those who had never taken the anthelmintic, and with Upatham et al. (1988) who showed that about 86% of those treated for OV infection were reinfected within one year. However, the finding is opposite to that reported in the study by Forrer et al. (2012), which found that prior treatment with praziquantel was a preventative factor for OV infection.

The association between OV infection and eating of unsafely prepared freshwater fish is well-established in Thailand and adjacent countries in the Lower Mekong Basin. The association with a similar dietary practice in the family is not surprising in terms of sharing meals with others in the same family and a society in which groups within the community people follow the same embedded cultural practices.

The indication that unsafe ways of waste food disposal are risk factor for OV infection appears to be a new finding. We have been unable to find any previous studies about this, and clearly it needs further exploration. If the finding is replicated, then at this stage we can only speculate that the association is mediated by a family's generally poor observance of health-promoting behaviour.

The findings of this study highlight the features on which a community programme should focus in order to reduce the prevalence of opisthorchiasis and incidence of bile duct cancer.

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Table 2. Association of Demographic and Other Factors with *Opisthorchis viverrini (OV)* Infection: Multivariate Analysis

Variables		Cases (n=117)	Controls (n=234)	OR_{crude}	OR_{adj}	95%CI of OR_{adj}	p-value
History of O. viverrini infection	Never	90	225	1	1	-	-
	Ever	27	9	7.5	5.64	2.10-15.18	0.001
History of taking praziquantel	Never	47	193	1	1	-	
	Ever	70	41	7.01	5.66	3.11-10.29	<0.001
Habit of eating unsafely prepared fish	Not eat	15	128	1	1	-	-
	Eat	102	106	8.21	5.17	2.49-10.74	<0.001
Habit of eating unsafely prepared fish by family members	Not eat	24	126	1	1	-	-
	Eat	93	108	4.52	3.25	1.63-6.49	0.001
Safe disposal of waste food	Yes	34	83	1	1	-	-
	No	83	151	1.34	2.04	1.10-3.80	0.02

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