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Incidence and Risk Factors of *Opisthorchis viverrini* Infections in a Rural Community in Thailand

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Abstract. *Opisthorchis viverrini* infection is one of the major public health problems in Thailand. Although information about transmission of this parasite to humans is well established, infections are still prevalent in the northern and northeastern Thailand. Thus, prospective epidemiologic information is needed for developing more effective public health interventions. A cohort study to identify incidence and risk factors of *O. viverrini* infection was conducted in a rural community in Thailand during December 2002–February 2004. The incidence rate of opisthorchiasis was 21.6/100 person-years. The independent factors associated with opisthorchiasis were an age > 60 years and consuming chopped raw fish salad (*Koi pla*). Thus, avoiding this raw fish salad should be emphasized in the national control program.

INTRODUCTION

Human liver fluke infections, opisthorchiasis is caused by *Opisthorchis viverrini* and *O. felineus*. Clonorchiasis is an infection with *Clonorchis sinensis*. These liver fluke infections remain a major public health problem in different areas. *Opisthorchis viverrini* infection is endemic in Southeast Asia, especially in Thailand, Lao PDR, Vietnam, and Cambodia; *O. felineus* infection is prevalent in eastern Europe. Countries in eastern Asia including China, Japan, Korea, Taiwan, and northern Vietnam are the endemic areas of *C. sinensis*.[1,2] In Thailand, approximately six million persons harboring *O. viverrini* live in the northern and northeastern regions.[3] *Clonorchis sinensis* infection was recently reported in central Thailand.[4] Chronic infection of *O. viverrini* may lead to cholangiocarcinoma.[5] The reported incidence of cholangiocarcinoma ranged from 93.8 to 317.6 per 100,000 person-years in Khon Kaen in northeastern Thailand, the area in which *O. viverrini* infection is endemic.[6] An epidemiologic survey in Thailand showed that the average prevalence of opisthorchiasis in 2001 was relatively high, ranging from 9.6% to 19.3%.[7] In addition, a study conducted in 2004 showed that the average prevalence of *O. viverrini* infection was 24.5%, ranging from 2.1% to 70.8% in various districts of Khon Kaen.[6]

Persons in these areas have consumed undercooked or raw animal products, in particular freshwater fish.[7–9] Although information about fish-borne transmission of *O. viverrini* is well established, the outcome of public health prevention and control programs for opisthorchiasis during the past decade has not been succesful.[7] Opisthorchiasis prevention campaigns focusing on avoiding the consumption of undercooked or raw freshwater fish may not be sustainably effective in Thailand. Prevalences have not been substantially reduced in highly endemic areas. Changing food consumption behaviors among the at-risk population is a difficult task in public health interventions. Several undercooked fish foods are still common among populations in northern and northeastern Thailand. Several reported risk factors showed a significant association of non-specific undercooked fish foods and acquiring *O. viverrini* infection. To develop more effective intervention strategies, information related to specific food consumption habits that increase the risk of acquiring opisthorchiasis is required.

Most studies of opisthorchiasis in Thailand were conducted using a cross-sectional study design, which may place limitations on obtaining unbiased risk factors. To obtain the incidence and associated risk factors of opisthorchiasis in this community, we conducted a cohort study was performed during December 2002–February 2004. The purpose of this study was to obtain information on risks associated with opisthorchiasis, including social behavior of people in the disease-endemic area.

MATERIALS AND METHODS

Study area and population. The study was conducted at Baan Nayao village in a rural area of Chacheongsao Province, Thailand, 228 km east of Bangkok. The population of the study area was 1,524 in 2004. The village is surrounded by ponds and reservoirs, which harbor snail and fish intermediate hosts of *O. viverrini*. Most of the population migrated from northeastern Thailand and has lived in this area for more than 30 years. They have maintained their northeastern-style habits of eating undercooked and raw freshwater fish.

The baseline survey of *O. viverrini* infection was carried out in December 2002. Of 668 stool specimens, 142 (21.3%) were positive for *O. viverrini* infection. Those persons who were negative for *O. viverrini* (n = 526, 78.7%) by stool examination were invited to be enrolled into a follow-up survey in February 2004 to identify incidence and risk factors of *O. viverrini* infection.

Stool collection and examination. At the baseline and follow-up surveys, a stool specimen from each participant was examined for intestinal parasites within 6 hours after collection in the field using normal saline wet preparation and Kato thick smear method.[10] Stool specimens were then examined under a light microscope for intestinal parasites, with an emphasis on *O. viverrini*. These specimens were then transported to the Department of Parasitology, Phramongkutklao College of Medicine, Bangkok, for the confirmation of *O. viverrini* infection by experienced technicians using formalin-ethyl acetate concentration technique.[11] *Opisthorchis viverrini* positivity was defined as the presence of *O. viverrini* eggs in the stool specimen examined by at least one of three methods: normal saline wet preparation, Kato thick smear, and formalin-ethyl acetate concentration technique.

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Questionnaires. Enrolled participants were interviewed using standardized questionnaires to identify the risk factors for *O. viverrini* infection. The questionnaire covered demographic data, socioeconomic conditions, sanitary behavior, including cooking and eating habits of several traditional fish dishes during the past 12 months, sources of fish for food preparation, sources and treatment methods of drinking water, and a history of animals contact. The questionnaire was also designed to cover traditional fish food items, which were grouped into three categories: uncooked fish, i.e., chopped raw fish salad (*Koi pla*); moderately fermented fish, i.e., briefly fermented fish (*Pla som*) and briefly fermented fish stuffed with rice (*Pla faak*); and completely fermented fish in a highly concentrated salt solution, i.e., raw fermented fish (*Pla ra*). The villagers' interview sessions were conducted as a process of the questionnaire development to ensure the validity of the questions asked. The questionnaire has been pre-tested and used in the other previous surveys and was found to be reliable in identifying the potential food consumption exposures.

Statistical analysis. Incidence rate per 100 person-years of observation were calculated for each category of demographic and potential risk factor variables. The association between potential risk factors and *O. viverrini* infection were assessed using Pearson's chi-square test for categorical variables. A 0.05 cut-off point was used for the *P* value and applied in all statistical analysis. Crude relative risk (RR) and 95% confidence intervals (CIs) were calculated for *O. viverrini* infection and each of the other variables in a bivariate analysis. Multivariate Poisson regression analysis was performed to obtain adjusted RR and 95% CIs. All analyses were conducted using STATA/SE for Windows version 9.2 (StataCorp LP, College Station, TX).

Ethical consideration and treatment. The research protocol was reviewed and approved by Ethical Committee of the Medical Department of the Royal Thai Army. Written informed consents were obtained from the enrolled participants or their parents for children participants after they read the information sheet and consent form. Free proper anthelminthic treatment and health education were provided for those who had intestinal parasitic infections. *Opisthorchis viverrini*–infected cases were treated with a single dose of praziquantel (40 mg/kg).

RESULTS

Study population. Of 526 enrolled *O. viverrini*-negative participants, 258 (49.0%) were males. Agriculture (80.0%) was the main occupation. A total of 57% had education levels less than primary school. Annual household income was obtained from each family in Thai baht and converted to U.S. dollar at the rate of 40 baht/U.S. dollar. More than 9 of 10 study participants had an annual household income below the national average. The proportion of raw or undercooked fresh-water fish eating habits were as follows: consumption of briefly fermented fish (67.5%), raw fermented fish (66.3%), chopped raw fish salad (52.4%), and briefly fermented fish stuffed with rice (16.8%).

Incidence of opisthorchiasis. Of 526 participants who were negative for *O. viverrini* infection at the baseline survey in December 2002, 317 (60.3%) were enrolled in a follow-up evaluation in February 2004. *Opisthorchis* eggs were identified in 83 (26.2%) persons. The Kato thick smear method detected *O. viverrini* egg in 68 specimens, and the formalin-ethyl acetate concentration technique identified additional 15 cases. The incidence rate of opisthorchiasis was 21.6/100 person-years.

Risk factors for opisthorchiasis. The prevalence of *O. viverrini* infection was significantly different when analyzed by age group ($P < 0.001$), education level ($P = 0.047$), and occupation ($P < 0.001$). There was no significant difference between sex and different household income groups (Table 1). Univariate analysis showed that those who consumed chopped raw fish salad (RR = 2.07, 95% CI = 1.31–3.28) and briefly fermented fish stuffed with rice (RR = 1.78, 95% CI = 1.03–2.97) had a higher risk of acquiring the infection (Table 2). In contrast, consuming raw fermented fish (*Pla ra*) was not associated with opisthorchiasis (RR = 0.97, 95% CI = 0.61–1.59).

Multivariate Poisson regression analysis showed that age and chopped raw fish salad consumption were independently associated with opisthorchiasis (Table 2). The participants who were in 20–39, 40–59, and greater than 60 years of age were 3.1 (95% CI = 1.1–8.2), 2.7 (95% CI = 1.0–7.4), and 4.1 (95% CI = 1.5–11.8) times at greater risk of acquiring the infection compared with those of less than 20 years of age. In addition, those who consumed chopped raw fish salad had a 1.9 times higher risk of acquiring opisthorchiasis (95% CI = 1.1–3.3) than those who did not consume chopped raw fish salad after adjusting for sex, age, education level, occupation, and a history of consuming other fish food items. There was no significant association with other traditional freshwater fish food.

DISCUSSION

Our study showed a relatively high incidence rate of *O. viverrini* infection of 21.6/100 person-years in the study community. The infection rate was similar to that reported in Khon Kaen Province (24%), an area in northeastern Thailand in which opisthorchiasis is highly endemic.[6,7,12] We used the formalin-ethyl acetate concentration technique, which is considered to be the most sensitive and reliable method for detection of *O. viverrini* eggs in confirming the results of the normal saline wet preparation and the Kato thick smear method in the baseline and follow-up surveys.[13] However, we examined only a single stool sample for each participant. Thus, those persons with light infection intensities might be missed. This finding might have affected the incidence rate. Another limitation of this study might be the relatively low follow-up rate (60.3%) among those who had negative stool examination results for *O. viverrini* dur-

TABLE 1

Incidence of *Opisthorchis viverrini*-infected persons and characteristics of enrolled subjects, Thailand

Characteristic	No. enrolled	No. (%) infected	*P*
Sex			
Male	141	43 (30.5)	
Female	176	40 (22.7)	0.125
Age group, years			
0–19	104	8 (8.0)	
20–39	54	17 (31.5)	
40–59	108	35 (32.4)	
≥ 60	51	23 (45.1)	< 0.001
Education level			
Primary school	246	71 (28.9)	
Above primary school	70	12 (17.1)	0.047
Family income (US$/years)			
0–500	158	55 (34.8)	
> 500	75	20 (26.7)	0.233
Occupation			
Agriculture	164	59 (36.0)	
Others	113	24 (15.7)	< 0.001

TABLE 2
Univariate and multivariate analysis of risk factors for acquiring *Opisthorchis viverrini* infection, Thailand*

Characteristic	No. infected	Person-years of follow-up	Incidence rate/ 100 person-years	Crude RR (95% CI)	Adjusted RR (95% CI)
Sex					
Female	40	214.1	25.2	1	1
Male	43	170.9	18.7	1.4 (0.9–2.1)	1.2 (0.8–1.9)
Age group, years					
0–19	8	127.6	6.3	1	1
20–39	17	66.3	25.7	4.1 (1.7–11.0)	3.1 (1.1–8.2)
40–59	35	130.4	26.8	4.3 (2.0–10.7)	2.7 (1.0–7.4)
≥ 60	23	60.7	37.9	6.0 (2.6–15.6)	4.1 (1.45–11.8)
Education level					
Higher than primary school	12	86.8	13.8	1	1
Primary school	71	298.2	23.8	1.7 (0.9–3.5)	0.9 (0.4–1.8)
Family income (US$/years)					
> 500	20	91.6	21.8	1	
0–500	55	191.9	28.7	1.3 (0.8–2.3)	
Occupation					
Others	24	186.0	12.9	1	1
Agriculture	59	199.1	29.6	2.3 (1.41–3.9)	0.9 (0.5–1.7)
Fish menus					
Chopped raw fish salad (*Koi pla*)					
No	39	230.27	16.9	1	1
Yes	43	122.5	35.1	2.1 (1.3–3.3)	1.9 (1.1–3.3)
Briefly fermented fish (*Pla som*)					
No	27	146.0	18.5	1	1
Yes	56	212.8	26.3	1.4 (0.9–2.3)	1.0 (0.6–1.7)
Raw fermented fish (*Pla ra*)					
No	28	118.7	23.6	1	
Yes	55	240.2	22.9	1.0 (0.6–1.6)	
Briefly fermented fish stuffed with rice (*Pla faak*)					
No	60	296.9	20.2	1	1
Yes	21	58.4	36.0	1.8 (1.0–3.0)	1.4 (0.8–2.3)

* Data were adjusted for sex, education level, and occupation. RR = relative risk; CI = confidence interval.

ing the 2002 study. The overall incidence rate might be overestimated to some extent because the incidences of *O. viverrini* infection were increased in the older age group. However, most persons who did not participate in the follow-up survey in 2004 were young adults who temporarily moved out of the village to find jobs during the dry season. However, the findings regarding risk factors would not be affected because the reasons for not participating in the study were unlikely to be related to food consumption behavior and *O. viverrini* infection.

Another potential bias might be related to the relatively long recall period (one year) of the food consumption history. Other previous studies using questionnaires for rapid identification of schistosomiasis in Africa and for risk factors of helminthic infections in China used a much shorter recall period, usually about four weeks.[14,15] In our study, however, we noted a high seasonal variation in consumption of uncooked food in this community, especially for chopped raw fish salad consumption that usually take place during the dry season. The questionnaire was designed to cover the food consumption over the whole previous year. In addition, because we collected the exposure information at the same time of stool specimen collections, our findings on risk factors were not likely to be compromised by information bias.

In our study, the incidence of opisthorchiasis was not significantly different between sexes but was different among age groups. The higher incidences were found in the older age groups compared with those less than 19 years of age. Using a multivariate Poisson regression model, we found that those who were greater than 60 years of age had an approximately four times greater risk of acquiring opisthorchiasis. This finding is in agreement with those of previous reports.[6,8] Although health education to avoid all kind of raw or undercooked fish food has been implemented in the study area, the older population still maintained their habits of eating undercooked fish. It is apparent that recommendations that the public avoid non-specific undercooked fish dishes have not been achieved, especially among the elderly. Other reasons might be that affected people feel less concerned about the infection because it is asymptomatic and an effective treatment against the parasites (praziquantel) is available. It has been demonstrated that there is an association between chronic infection with *O. viverrini* and cholangiocarcinoma. Persons 65–69 years of age with chronic infections with opisthorchiasis were 2.5 times more likely to show development of cholangiocarcinoma than other age groups.[6] Thus, health education regarding the threat of chronic opisthorchiasis needs to be continuously communicated to affected people. Because the older population seemed to have difficulties in changing its eating habits, health education programs to avoid raw and undercooked fish foods should be focused on young persons who would be more likely to change their behavior.

Using a cross-sectional approach, Upatham and others found that more than 90% of heavily infected persons had a history of eating chopped raw fish salad compared with 19% of uninfected persons.[8] Recently, a study of opisthorchiasis in Lao PDR also showed that persons consuming raw or undercooked fish had a 2.3 times greater risk of acquiring the infection.[16] From these studies, it was concluded that eating preference, especially raw or undercooked fish dishes, enhances the risk of being infected with *O. viverrini*. However, eating undercooked fish and actual risks of acquiring the infection were not demonstrated in this study. Our study was the first cohort study of

O. viverrini infection conducted in Thailand that identified the risks for *O. viverrini* infection.

There are at least 15 species of small and medium-sized cyprinoid fish that naturally harbor the infective metacercariae, and these fish being the source of infection.[17,18] More than 10 popular raw or undercooked fish dishes had been prepared and consumed throughout the year by local persons at the study site. Of the four favorite dishes of freshwater fish, multivariate Poisson regression analysis confirmed that only chopped raw fish salad consumption was significantly associated with opisthorchiasis; the others were not. Persons who consumed chopped raw fish salad had a 1.9 times greater risk of acquiring the infection than those who did eat this salad after adjusting for sex, age, education level, occupation, and a history of consuming other fish food items.

On the basis of informal interviews with villagers, frequencies of eating chopped raw fish salad have been decreased and consumption generally was confined to special social occasions in this community, such as the fish catching season in summer. During this season, chopped raw fish salad was freshly prepared, and then immediately consumed near the ponds. This information was also reported in other studies.[1] In this community, fully preserved fish, i.e., raw fermented fish, was regularly consumed several times a week all year round. This preserved fish was also consumed daily by most people in opisthorchiasis-endemic areas in northeast Thailand.[19] Nevertheless, we found that raw fermented fish were not associated with opisthorchiasis. Preparation of raw fermented fish was unique because it was kept fermented in a highly salted condition for at least 3–6 months. Survival of metacercaria depends on the concentration of salt and duration of fermentation.[20] Sukonthason and others reported that metacercariae in briefly fermented fish were degenerated after the second day.[21] Our interviews showed that raw fermented fish was prepared by local people and kept at least six months. Thus, it is most likely that metacercariae in raw fermented fish were non-pathogenic to humans. The present data showed that raw fermented fish was safe to consume because it was not the potential risk of *O. viverrini* infection in this community.

In conclusion, the national opisthorchiasis control program in Thailand should clearly address the threat of chopped raw fish salad consumption in acquiring *O. viverrini* infection. Strategic approaches for the opisthorchiasis control program should include stool examination and treatment of parasite-positive cases with praziquantel to eliminate human host reservoirs. Health education that emphasizes avoiding raw freshwater fish consumption, especially chopped raw fish salad, for preventing *O. viverrini* infection should be implemented.

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