RESEARCH ARTICLE

Prevalence of *Opisthorchis viverrini* Infection in Nakhon Ratchasima Province, Northeast Thailand

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Abstract

Background: *Opisthorchis viverrini* infection is a serious public-health problem in Southeast Asia especially in Lao PDR and Thailand. It is associated with a number of hepatobiliary diseases and the evidence strongly indicates that liver fluke infection is the major etiology of cholangiocarcinoma. Objectives: This study aimed to determine actual levels of *Opisthorchis viverrini* infection in Nakhon Ratchasima province, Northeast Thailand. Methods: A cross-sectional survey was conducted during a one year period from October 2010 to September 2011. *O. viverrini* infection was determined using a modified Kato's thick smear technique and socio-demographic data were collected using predesigned semi-structured questionnaires. Results: A total of 1,168 stool samples were obtained from 516 males and 652 females, aged 5-90 years. Stool examination showed that 2.48% were infected with *O. viverrini*. Males were slightly more likely to be infected than females, but the different was not statistically significant. *O. viverrini* infection was most frequent in the 51-60 year age group and was found to be positively associated with education and occupation. Positive results were evident in 16 of 32 districts, the highest prevalence being found in Non Daeng with 16.7%, followed by Pra Thai with 11.1%, Kaeng Sanam Nang with 8.33%, and Lam Ta Men Chai (8.33%) districts. Conclusion: This study indicates that *O viverrini* is still a problem in some areas of Nakhon Ratchasima, the patients in this study bing suitable for the purpose of monitoring projects.

Keywords: Prevalence - carcinogenic human liver fluke - *opisthorchis viverrini* - Thailand

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Introduction

Opisthorchiasis caused by *Opisthorchis viverrini*, is of considerable public health importance in Southeast Asia, particularly in Lao PDR and Thailand (Sripa et al., 2010). Human have been infected by ingesting undercooked fish containing infective metacercariae, this is very common in the northeastern and northern region particularly in rural areas (Sadun, 1955; Wykoff et al., 1965; Vichasri et al., 1982; Sithithaworn et al., 1997; Jongsuksuntigul and Imsomboon, 2003). The infection is associated with a number of hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis and cholelithiasis (Harinsuta and Vajrasthira, 1960; Harinasuta et al., 1984). The experimental and epidemiological evidences strongly indicate that the liver fluke infection in the etiology of cholangiocarcinoma (CCA); the bile duct cancer (Thamavit et al., 1978; IARC, 1994; Sripa et al., 2007).

In Thailand, it is estimated that 6 million people are infected with the *O. viverrini* (Sithithaworn et al., 2012). The first nationwide survey of the four regions of Thailand during 1980-1981 revealed an overall prevalence of *O. viverrini* infection of 14%; the Northeast (34.6%), the Central (6.3%), the North (5.6%) and the South (0.01%) regions (Jongsuksantikul and Imsomboon, 2003). As a result of intensive and continuous control programs and public health service activities, the average national prevalence of infection has declined to 9.4% in the year 2000 and went down further to 8.7% in the year 2009 (Jongsuksantikul and Imsomboon, 2003). Again, high prevalence of the infection was found in the Northeast (16.6%) followed by the North (10.0%), the Central (1.3%) and the South (0.01%) region of Thailand. Indeed, in the Northeast Region the prevalence in 2009 was similar to that of the previous survey 10 years ago in the year 2000 (15.7%) (Sithithaworn et al., 2012). *O. viverrini* infection in Thailand, particularly in the North and Northeast Regions, is still prevalent and the highest in the world, however, no detailed data on its prevalence in population from Nakhon Ratchasima province, Thailand have been reported. Therefore, a community-based cross-sectional study was conducted among villagers in Nakhon Ratchasima province, Thailand. This research data could be used to localize the risk areas to prevent and control the infection.

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Materials and Methods

Epidemiological data were obtained from a community-based survey conducted from October 2010 to September 2011 from 32 districts of Nakhon Ratchasima province, Thailand. A cross-sectional parasitological and questionnaire survey was carried out in Nakhon Ratchasima province located in northeastern with the total area about 20,494 square kilometres, making it the biggest province in Thailand, 259 kilometres away from Bangkok city by cars. The province is subdivided into 32 districts (amphoe). The districts are further subdivided into 263 sub-districts (tambon) and 3,743 villages (muban). Mueang Nakhon Ratchasima, Khon Buri, Soeng Sang, Khong, Ban Lueam, Chakkarat, Chok Chai, Dan Khun Thot, Non Thai, Non Sung, Kham Sakaesaeng, Bua Yai, Prathai, Pak Thong Chai, Phimai, Huai Thalaeng, Chum Phuang, Sung Noen, Kham Thale So, Sikhio, Pak Chong, Nong Bun Mak, Kaeng Sanam Nang, Non Daeng, Wang Nam Khiao, Thepharak, Mueang Yang, Phra Thong Kham, Lam Thamenchai, Bua Lai, Sida, and Chaloem Phra Kiat, respectively.

The study protocol was approved by Suranaree University Ethical Review Committee (2009). A total of 1,168 villagers, 516 male and 652 female were randomly selected. Necessary permission from the concerned authorities was taken and a survey was conducted using pretested semi-structured questionnaires. Prior informed consent was taken. For those not available in the first interview another visit was made to minimize non response. Stools were collected from individualized villager (who had completed the interviewed) and kept in labeled plastic bags and then transported in the box to the laboratory at the Parasitic Disease Research Unit, department of Pathology, Institute of Medicine, Suranaree University of Technology, Thailand, within a day after collection.

A total of 1,168 stool specimens were collected and examined the *O. viverrini* by the modified Kato thick smear procedures. The modified Kato thick smear was prepared and processed according to the method of Kato and Miura (1954). The materials used were prepared in accordance with standard laboratory in-house procedures. Thus, the glycerin-malachite green solution was mixed with 1 ml of 3% malachite green, 100 ml of 6% phenol and 100 ml of pure glycerin. The cellophane strips, each 22x40 mm, were soaked in this solution for at least 24 hours before use. Additionally, in order to eliminate fibers or seed, the technique was modified by pressing a 105-mesh stainless steel grid onto the sample which was then filtered, transferred to slides covered by the cellophane soaked cover slips and allowed to stand for 30 minutes. All preparations were initially screened with a low-power (10x) objective lens. Suspected parasitic objects were subsequently examined under a high-power (40x) objective. The stool samples were preserved in 10% formalin for later confirmation, if needed. Every positive case of *O. viverrini* infection identified by the modified Kato method was confirmed by 2 exerted parasitologists before a definitive diagnosis was established. O. viverrini egg was shown in Figure 1. Patients who infected with other known parasitic were treated with anti-parasitic drugs and also attended the health education.

Statistical data analysis was carried out using SPSS software version 12.0. Chi-square test was performed to determine association between socio-demographic and *O. viverrini* infection.

Results

The survey results are summarized in Tables 1 to 3. A total of 1,168 villagers (55.82% females and 44.18% males) was participated the survey. The sample

Figure 1. The *O. viverrini* egg was determined from the Elderly Selected from Various 17 Districts, Surin Province, Thailand (Magnitude 1000x)

Table 1. *O. viverrini* infection in villagers from Nakhon Ratchasima, Thailand by gender, age, education, and occupation

Sex	Male	2.97%	(15/516)
	Female	2.15%	(14/652)
Age	12-May	0	(0/62)
	13-20	2.13%	(1/47)
	21-30	1.96%	(1/51)
	31-40	2.58%	(4/155)
	41-50	2.66%	(12/451)
	51-60	2.75%	(6/218)
	61-70	1.56%	(2/128)
	70-80	6.12%	(3/49)
	80-90	0	(0/7)
Education Level	Primary	3.05%	(27/886)*
	Secondary	0.93%	(2/214)
	College	0	(0/68)
Occupation	Agriculture	3.76%	(23/612) *
	Employed	1.26%	(4/317)
	House work	1.40%	(2/143)
	Trade	0	(0/52)
	Government officer	0	(0/36)
	Beautician	0	(0/8)
Total		2.48%	(29/1168)

*p value=0.05

Table 2. Intestinal Parasitic Infection in villagers from Nakhon Ratchasima, Thailand by species

Parasitic species	% (No. of patient infected/examined)
Opisthorcris viverrini	2.48 (29/1168)
Taenia spp.	0.68 (8/1168)
Strongiloides stercoralis	0.43 (5/1168)
Hookworm	0.34 (4/1168)
Giardia lamblia	0.34 (4/1168)
Trichuris trichiura	0.17 (2/1168)
Ascaris lumbricoides	0.17 (2/1168)
Haplochis pumilio	0.09 (1/1168)
Echinostome sp.	0.09 (1/1168)
Total	4.79 (56/1168)

Table 3. *O. viverrini* infection in Nakhon Ratchasima, Northeast Thailand by Districts

Districts	%(No. of patient infected/examined)
Non Daeng	16.67 (6/36)
Pra Thai	11.11 (4/36)
Lam Ta Men Chai	8.33 (3/36)
Kaeng Sanam Nang	8.33 (3/36)
Huai Thalaeng	5.50 (2/36)
Chaloem Phra Kiat	2.78 (1/36)
Chakkarat	2.78 (1/36)
Dan Khun Thot	2.78 (1/36)
Non Thai	2.78 (1/36)
Mueang Yang	2.78 (1/36)
Chum Phuang	2.78 (1/36)
Non Sung	2.78 (1/36)
Bua Lai	2.78 (1/36)
Sida	2.78 (1/36)
Khon Buri	2.5 (1/40)
Mueang Nakhon Ratchasima	2.5 (1/40)
Soeng Sang	0 (0/40)
Chok Chai	0 (0/36)
Pak Thong Chai	0 (0/40)
Sung Noen	0 (0/36)
Khan Thale So	0 (0/36)
Sikhio	0 (0/36)
Pak Chong	0 (0/36)
Nong Bun Mak	0 (0/36)
Wang Nam Khiao	0 (0/36)
Thepharak	0 (0/36)
Phra Thong Kham	0 (0/36)
Phimai	0 (0/36)
Kaham Sakaesaeng	0 (0/36)
Khong	0 (0/36)
Ban Lueam	0 (0/36)
Bua Yai	0 (0/36)
Total	2.48 (29/1168)

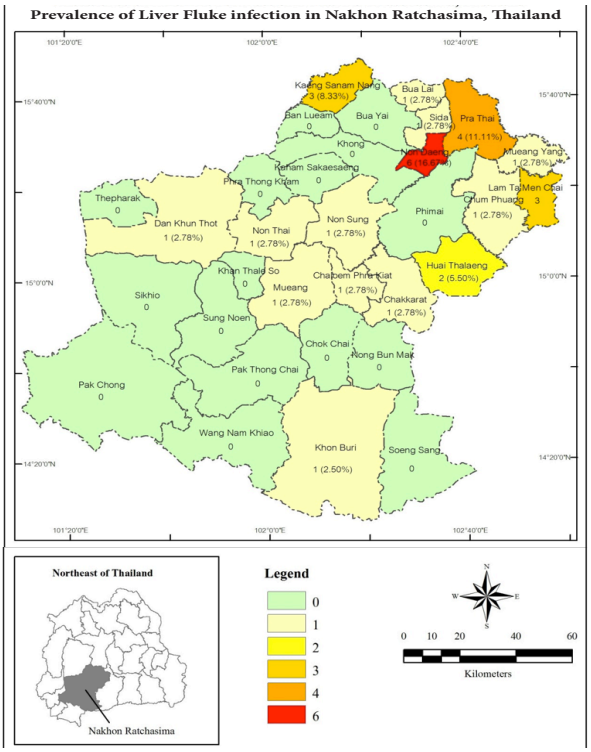

Figure 2. The Prevalence of *O. viverrini* infection in villagers from Nakhon Ratchasima Province, Northeast Thailand

represented 38.61% of the total population of the majority 41-50 year age group. Education level, a majority of population was the primary level (99.06%), and they were a farmer (75.86%). General characteristics of population were shown in Table 1. The stool examination results showed that 2.48% of the participants were infected with *O. viverrini*. Other known intestinal parasitic infections were *Taenia sp.* (0.68%), *Strongiloides stercoralis* (0.43%), hookworm (034%), *Giardia lamblia* (0.34%), *Trichuris trichiura* (0.17%), *Ascaris lumbricoides* (0.17%), *Haplochis pumilio* (0.09%), *Echinostome sp.* (0.09%), respectively (Table 2). The proportion of infected males (2.97%) was slightly higher than the infection rate in females (2.15%), but the gender difference was not statistically significant, X^2 (1, N=1,168)=1.63, p=0.35. The prevalence of *O. viverrini* infection frequently with 51-60 year age group (2.75%) and educational level of primary level (3.05%) (Table 1). Chi-square testing was analyzed on the education and occupation, a significant associated between primary level (X^2 (2, N=886)=8.32, P=0.05) and farmer (X^2 (3, N=612)=9.74, P=0.05) and *O. viverrini* infection. The infection rates of *O. viverrini* in 32 districts for the year-round survey are shown in Table 3 and Figure 2. The distribution of *O. viverrini* in Nakhon Ratchasima areas was analyzed and found that patients infected with *O. viverrini* in 16 of 32 districts. The highest prevalence was found in Non Daeng with 16.67%, and followed by Pra Thai (11.11%), Kaeng Sanam Nang (8.33%), and Lam Ta Men Chai (8.33%) districts, respectively.

Discussion

The Helminthiasis control program started in 1950 included opisthorchiasis control in some high risk areas. The main liver fluke control strategies comprise of three interrelated approaches, namely stool examinations and treatment of positive cases with praziquantel for eliminating human host reservoir; health education for a promotion of cooked fish consumption to prevent infection, and the improvement of hygienic defecation for the interruption of disease transmission (Jongsuksuntigul and Imsomboon, 2003). The first nationwide survey of the four regions of Thailand during 1980-1981 revealed an overall prevalence of *O. viverrini* infection of 14%; the Northeast (34.6%), the Central (6.3%), the North (5.6%) and the South (0.01%) regions. As a result of intensive and continuous control programs and public health service activities, the average national prevalence of infection has declined to 9.4% in the year 2000 and went down further to 8.7% in the year 2009 (Jongsuksantikul and Imsomboon, 2003; Sithithaworn et al., 2012). Based on the data in 2009, *O. viverrini* infection in Thailand, particularly in the North and Northeast Regions, is still prevalent and the total number of opisthorchiasis cases is estimated to be more than 6 million, the highest in the world (Sithithaworn et al., 2012). Although, the helminthiasis control program have been started for a long time but it still prevalent and found the *O. viverrini* in human and rural community.

This study represents a prospective 'work in progress'

and is intended to provide baseline information for future follow-up surveys to monitor infection (and especially re-infection in the population age group) rates. The report on the next survey will include detailed the participants information about their knowledge, beliefs and behaviors relevant to *O. viverrini* infection. The current prevalence rates in population from community Nakhon Ratchasima are low. This rate indicates that the results of the national control program in rural parts of Northeast Thailand are appointing, the program does appear to be having much impact in these areas. The results of the present study suggest a slight, but non-significant higher prevalence of *O. viverrini* infection in male community-dwellers than in females. There was also a strong positive correlation with age in both males and females, especially in males. Analyses of data from annual surveillance of Thailand and some community surveyed have shown a higher prevalence of *O. viverrini* infection in males than females (Upatham et al., 1982; Kaewpitoon et al., 2008). Based on the data from Khon Kaen cohort study have shown a higher prevalence of *O. viverrini* infection in males than females and increasing prevalence with age in females, but not in males (Sriamporn et al.,2004). In the present study, prevalence was frequently with 41-50 and 51-60 age groups. Studies of village communities elsewhere in Thailand have reported results consistent with the population Nakhon Ratchasima data. Rhongbutsri and Kitvatanachai (2002) found prevalence was highest in villagers over 50 years old, and the results of Rangsin et al. (2009) also showed increasing rates of infection with age. While the higher rates of infection in the older age groups puts these people at greater risk of cholangiocarcinoma and can result in serious losses of income for families when they develop the disease during their working lives.

This study represents the education for the primary level was a frequently with *O. viverrini* infection. This figure indicates less knowledge due to *O. viverrini* infection in the villager community. Kaewpitoon et al. (2007) showed a lower educational level related to liver fluke infection in northeastern Thailand. In the present study, occupation was significant associated between farmers with *O. viverrini* infection. This result was similar to that previous studied about the knowledge related to liver fluke infection in Thailand. They may have a low knowledge and lack of data information on the *O. viverrini* transmission, prevention and control (Kaewpitoon et al., 2007; Kaewpitoon et al., 2008). In further studies, we should be following the patients who infected with *O. viverrini* about their knowledge, attitude, and practice.

The distribution of *O. viverrini* in Nakhon Ratchasima areas was found in 16 of 32 districts, the highest prevalence was found in Non Daeng, and followed by Pra Thai, Kaeng Sanam Nang, and Lam Ta Men Chai districts. Unfortunately, lack of data on their knowledge, attitude, and practice related to assess the risk factors of *O. viverrini* infection. However, we expect that they may have a behavior similarly to other villager who infected with *O. viverrini* in epidemicity areas. In addition, the highest prevalence areas of Nakhon Ratchasima located near Khon Kaen province, Northeast Thailand where have been reported a high incidence of *O. viverrini* and cholangiocarcinoma (Sripa et al., 2010; Sithithaworn et al., 2012), therefore the patient for study is suitable for the purpose of the monitoring project.

We have examined for intestinal parasitic infections and found that *Taenia sp., S. stercoralis, hookworm, G. lamblia, T. trichiura, A. lumbricoides, H. pumilio, Echinostome sp.,* infected in population. Although this data show a small scale of prevalence in this area however this study indicates that parasitic infection is still a problem in some community and need to improve their health education. Some parasites can induce a chronic disease for a long time infection. Hookworm larvae can enter through the skin of host, typically on the foot, and travel up through the bloodstream into the lungs. Hookworm disease can produce an itchy rash, coughing with or without bloody sputum. When present in the intestines, hookworm infection usually does not produce any recognizable symptoms. However, some people may experience diarrhea, abdominal pain, intestinal cramps, and nausea. Chronic or persistent hookworm can cause anemia due to blood loss, especially in people with poor health or in pregnant women (Ball and Michael, (1991).

In conclusion, our study showed the first report on the prevalence of *O. viverrini* in population from Nakhon Ratchasima Province, Thailand, therefore, cholangiocarcinoma diagnose, health educations are urgently required, and the patient for study is suitable for the purpose of the monitoring project.

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