RESEARCH ARTICLE

Re-Examination of *Opisthorchis viverrini* in Nakhon Ratchasima Province, Northeastern Thailand, Indicates Continued Needs for Health Intervention

Soraya J Kaewpitoon[1,2,3]*, Ratana Rujirakul[1], Ryan A Loyd[2,3], Sukij Panpimanmas[3,4], Likit Matrakool[3,4], Taweesak Tongtawee[3,4], Porntip Kompor[5], Jun Norkaew[5], Wasugree Chavengkun[5], Jirawoot Kujapan[5], Sukanya Polphimai[5], Tanida Phatisena[6], Thawatchai Eaksunti[6], Poowadol Polsripradist[7], Natnapa Padchasuwan[8], Natthawut Kaewpitoon[1,3,5]

Abstract

***Opisthorchis viverrini* infection is associated with cholangiocarcinoma particularly in the cases of chronic or re-infection. This presents a serious health problem in northeastern and northern Thailand. A community base approach is required for surveillance. Therefore, in a pilot project, re-examination of *O. viverrini* infection was conducted in the 3 districts of Nakhon Ratchasima province, Thailand, during June and October 2015. A total of 355 participants from a 194,152 population, was selected through multi-stage sampling. *O. viverrini* infection was determined using modified Kato Katz thick smear technique. Participants were 229 males and 126 females, and aged ≥30 years old. Prevalence of *O. viverrini* infection was 2.25% (8/355 participants). *O. viverrini* infection was slightly higher in females (3.17%), and age group between 41-50 years (4.49%). Mueang Yang district had a highest of *O. viverrini* infection rate (2.82%), and followed by Bua Yai (2.48%), and Chum Phuang (1.84%), respectively. *O. viverrini* infection rate was increased from year 2012 to 2015 particularly in Bua Yai and Mueang Yang. These re-examinion results indicate that opisthorchiasis is still problem in community of Nakhon Ratchasima province, therefore, the provincial-wide scale is need required. Furthermore health education is need intervened in the infected group, and screening of cholangiocarcinoma is urgently concerned.**

Keywords: Re-examination - *Opisthorchis viverrini* - cholangiocarcinoma - Nakhon Ratchasima - Thailand

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Introduction

The *Opisthorchis viverrini*; carcinogenic liver fluke, is an endemic in the Lower Mekong Basin, including Thailand, Lao People's Democratic Republic, Cambodia and central Vietnam (Sripa et al., 2010). The under-estimate of infections are considered, more than 10 million people are infected with *O. viverrini* in Thailand and Lao PDR (Sithithaworn et al., 2012). In Thailand, it is estimated that 6 million people are infected with the *O. viverrini* (Jongsuksuntikul and Imsomboon, 2003). This figure indicated that it is a serious public health problem in Thailand, particularly in northeastern and northern region (Kaewpitoon et al., 2008; Sripa et al., 2010). The *O.viverrini* infection is associated with hepatobiliary diseases including hepatomegaly, cholangitis, cholecystitis, and gallstones (Harinasuta and Vajrasthira 1960; Thamavit et al., 1978; Harinasuta et al., 1984). Recently, *O. viverrini* has been classified as Type 1 carcinogens by the International Agency for Research on Cancer, World Health Organization (WHO) (IARC, 1994).

A community-level health education campaign been conducted since late 1950s. *O. viverrini* control has been started as a small scale helminthiasis control program in some high risk areas. A large scale has been started, the program is operated in some provinces of the central and all provinces of the northeast and north of Thailand. The main strategies for liver fluke control comprise three interrelated approaches, namely stool examination 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 improvement of hygienic defecation for the interruption of disease transmission (Jongsuksantikul and

*[1]Parasitic Disease Research Unit, [2]School of Family Medicine and Community Medicine, [3]Suranaree University of Technology Hospital, [4]School of Surgery, Institute of Medicine, Suranaree University, [5]Faculty of Public Health, Vongchavalitkul University, [6]Faculty of Public Health, Nakhon Ratchasima Rajabhat University, [7]Provincial Public Health of Nakhon Ratchasima, [8]Faculty of Public Health, Khon Kaen University, Khon Kaen, Thailand *For correspondence: soraya.k@sut.ac.th*

Imsomboon, 2003; Sithithaworn et al., 2012).

The *O.viverrini* infection in Thailand was the first reported in 1955 (Sadun) and many strategies has been operated over period 1955-2000, the national prevalence of *O. viverrini* infection had fallen from 63.6% to 9.6% but the high prevalence rate is still found in the rural communities of provinces, Northeast (Sithithaworn et al., 2012). In addition, the high mortality rate of CCA was reported in the northeast areas where found frequently of *O. viverrini* infection (Sripa et al., 2010). Mortality rate of liver cancer and *O. viverrini* infection rate in different regions of Thailand has been reported and found that Nakhon Ratchasima province has 13.67-16.2 per 100,000 populations. Eradication of the fluke and identification of high-risk populations are urgently needed (Sripa and pairojkul, 2008). In addition, the distribution *O. viverrini* infection in Nakahon Ratchasima province has been reported, the prevalence of survey in 2009 was 4.6% (Sitthithaworn et al., 2012). 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*. (Kaewpitoon et al., 2012c). Furthermore, a total of 640 Cyprinidae family fish including 5 species were collected from different study sites of Nakhon Ratchasima province, and investigated for *O. viverrini* metacercariae. The infection rate was 12.3% (79/640), predominantly in *Cyclocheilichthys armatus*, *C. repasson*, *Puntioplites proctzysron*, *Hampala macrolepitota* and *Hampala dispar*, respectively. The prevalence of *O. viverrini* metaceria was found covered 78.1% of areas, predominantly in Sida and KiaKham Thale So (Kaewpitoon et al., 2012a).

These figure indicate that *O. viverrini* infection is still a problem in this areas, a community-based approach to screen *O. viverrini* in highly risk areas are need required. Therefore, this study aimed to re-examine *O. viverrini* in 3 districts of Nakhon Ratchasima province using multi-stage sampling technique, between June and October 2015. This data is able useful for further therapy, curable, and planning of prevention and control.

Materials and Methods

A cross-sectional survey was a pilot project and conducted in 3 districts of Nakhon Ratchasima province, northeastern Thailand, between June and October 2015, included Bua Yai, Chum Phuang, and Mueang Yang district (Figure 1). Bua Yai is a district in the northern part of Nakhon Ratchasima, and neighboring districts are (from the north clockwise) Waeng Noi district of Khon Kaen province, Bua Lai, Sida, Non Daeng, Khong, Ban Lueam, and Kaeng Sanam Nang of Nakhon Ratchasima Province. Bua Yai district is subdivided into 10 sub-districts, and 121 villages. This district is coverage areas 271.6 km^2 (104.9 sq mi), and has 84,133 populations. Chum Phuang is a district in the eastern part of Nakhon Ratchasima province, neighboring districts are (from the north clockwise) Prathai, Mueang Yang and Lam Thamenchai of Nakhon Ratchasima Province, Lam Plai Mat of Buriram province, and Huai Thalaeng and Phimai of Nakhon Ratchasima again. The district is subdivided into 9 sub-districts, and 130 villages. This district is coverage areas 540.6 km^2 (208.7 sq mi), and has 82,161 populations. Mueang Yang is a district in the northeastern part of Nakhon Ratchasima province, neighboring districts are (from the north clockwise) Ban Mai Chaiyapot, Phutthaisong and Kho Mueang of Buriram province, Lam Thamenchai, Chum Phuang and Prathai of Nakhon Ratchasima Province. The district is subdivided into 4 sub-districts, and 44 villages. This district is coverage areas 255.5 km^2 (98.6 sq mi), and has 28,359 populations. The main water resource is the Mun river.

Multi-stage sampling was used to select the participants in this studied. Briefly, total of 66,163 populations from 194,152 populations was selected with criteria of aged ≥30 years old. Populations at risk were screened by using mini-verbal screening questionnaire contained the history with (1) opisthorchiasis; definitive diagnosed by medical doctor or related officers, (2) under-cooked fish consumption, (3) praziquantel used; given by medical doctor or related officers, (4) cholecystitis; definitive diagnosed by medical doctor or related officers, (5) relative family with

Figure 1. Map of Nakhon Ratchasima province, Northeastern Thailand; blue color lines are 3 districts of study areas including Bua Yai, Meuang Yang, and Chum Phuang (Adapted from http://www.novabizz.com/Map/img/map-36-Nakhonratchasima.gif)

Table 1. Baseline characteristics of participants in 3 districts of Nakhon Ratchasima province, Thailand.

Characteristics	No. n(%)	No. of infection n(%)
Gender		
Male	229(64.51%)	4(1.75%)
Female	126(35.49%)	4(3.17%)
Age		
30-40	29(8.17%)	0
41-50	89(25.07%)	4(4.49%)
51-60	130(36.62%)	2(1.54%)
61-70	98(27.61%)	2(2.04%)
>70	9(2.54%)	0
District		
Bua Yai	121(34.08%)	3(2.48%)
Chum Phuang	163(45.92%)	3(1.84%)
Mueang Yang	71(20%)	2(2.82%)
Total	355(100%)	8(2.25%)

cholangiocarcinoma, (6) naïve northeastern people, (7) agriculture, and (8) alcohol consumption. Population at risk was identified following 1+2+3+4+5+6+7+8, who had a high score more than 5 points was selected, included 71, 121, and 163 participants from Mueang Yang, Bua Yai, and Chum Phuang district (Figure 2). Stools were collected and kept in labeled plastic bags and then transported in an icebox to the Clinical Pathological laboratory at the Suranaree University of technology Hospital, Suranaree University of Technology, Thailand, within a day after collection. Stool specimens were examined the *O. viverrini* and other known intestinal parasitic egg by the Modified Kato Katz thick smear procedures. Briefly, a small amount of stool material was placed on scrap paper and a piece of nylon sieve was pressed on top of it so that some of the stool sieved through the screen and accumulated on top of the 180 mesh nylon sieve. A spatula was scraped across the upper surface of the screen to collect the sieved stool. The spatula was used to deposit the stool in the orifice of the perforated plate on a microscope slide. The perpendicular 4x10x1 mm orifice was devised to contain exactly 0.04 g or 40 μL of stool, at one corner of the slide. The other side of the spatula was passed over a nylon sieve and over the perforated plate to remove excess stool. The plate was carefully removed by lifting, leaving behind a small square mould of sieved material. Opposite corner of other microscope slide was pressed on top of this, and a relatively thin smear was obtained by sliding over the slides in order to provide a thick smear in each slide (Meireles et al., 2008). 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. *O. viverrini* positive case was confirmed by 2 expert parasitologists before a definitive diagnosis was established. Patients who infected with other known parasitic were treated with anti-parasitic drugs and also attended the health education. The data was analyzed with descriptive statistics. Statistical data analysis was carried out using SPSS software version 12.0. The study protocol was approved by Suranaree University Ethical Review Committee, EC58-48.

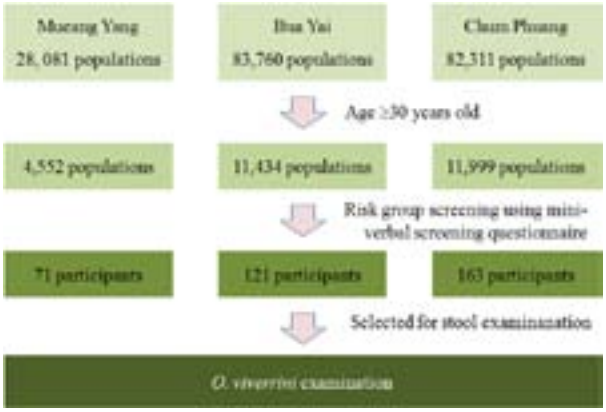

Figure 2. Diagram of Participant Selection in 3 Districts of Nakhon Ratchasima Province, Thailand, Using Multi-stage Sampling Technique

Figure 3. *O. viverrini* infection in 3 districts of Nakhon Ratchasima province, Thailand between 2012 and 2015

Table 2. *O. viverrini* infection in 3 districts of Nakhon Ratchasima province, Thailand, between 2012 and 2015.

Characteristics	2012		2015	
	No.	No. of infection n(%)	No.	No. of infection n(%)
District				
Bua Yai	36	0	121	3(2.48%)
Chum Phuang	36	1(2.78%)	163	3(1.84%)
Mueang Yang	36	1(2.78%)	71	2(2.82%)
Total	108	2(1.85%)	355	8(2.25%)

Results

Total of 355 participant from 3 districts, was included in this study. The prevalence of *O. viverrini* infection was 2.25%. *O. viverrini* infection was slightly in female (3.17%) more than male (1.75%). The majorities of *O. viverrini* infection were found in age between 41-50 years old (4.49%), and followed by 61-70 years old (2.04%), 51-60 years old (1.54%), respectively. *O. viverrini* infection was found the highest in Mueang Yang district (2.82%), and followed by Bua Yai (2.48%), and Chum Phuang (1.84%), respectively. Baseline characteristics and infection are shown in Table 1. Other known parasitic infections were examined and found that two samples were infected Blastocystis hominis, and one sample was infected with Strongyloides stercolaris. Patients who infected with *O. viverrini* and other known intestinal parasitic were completed therapeutic treatment. *O. viverrini* infection in 3 districts between the surveyed data in year 2012 (Kaewpitoon et al., 2012c) and 2015, was considered. Infection rate was increased in Bua Yai (0%) and Mueang Yang (2.78%) district in year 2012 to 2.48%, and 2.82%, in year 2015. In the opposite, *O. viverrini* infection was slightly decreased rate in 2015 (Table 2 and Figure 3).

Discussion

Opithorchiasis is still a serious health problem in Nakhon Ratchasima province, Thailand. Based on data was surveyed in 2009 and 2012, *O. viverrini* infection was decreased. *O. viverrini* infection was surveyed in 2009 and found that Nakhon Ratchasima province had 4.6%

(Sitthithaworn et al., 2012). Meanwhile, a provincial-wide surveyed in 2012 by Kaewpitoon et al (2012c) and found that 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*. However, identification of *O. viverrini* in the district scale and found that the infection rate was 2.78%, 2.78%, and 0% in Mueang Yang, Chum Phuang, and Bua Yai district. In addition, recent re-examined results were slightly increased in Mueang Yang (2.82%) and Bua Yai (2.48%) district in year 2015. Infection rate of *O. viverrini* in 3 districts in 2012 was 1.85%, while in 2015 was 2.25% of prevalence or morbidity rate = 2,250 per 100,000 population. This result indicates that *O. viverrini* infection is still a health problem in rural communities, and they are a risk group of cholangiocarcinoma. Recently we have known that the *O.viverrini* infection is associated with hepatobiliary diseases including hepatomegaly, cholangitis, cholecystitis, and gallstones (Harinasuta and Vajrasthira 1960; Thamavit et al., 1978; Harinasuta et al., 1984). In addition, *O. viverrini* has been classified as Type 1 carcinogens by the International Agency for Research on Cancer, World Health Organization (WHO) (IARC, 1994). Previously, the mortality rate of cholangiocarcinoma was reported and found that Nakhon Ratchasima province has 13.67-16.2 per 100,000 populations (Sripa and pairojkul, 2008). This figure indicates that Nakhon Ratchasima province should be intervened the health behavioral change particularly in these highly risk group. Furthermore, a provincial wide survey is need required, and screening of cholangiocarcnioma in infected participant and risk group is urgently concerned.

Acknowledgements

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