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

Soraya J Kaewpitoon1,2,3*, Ratana Rujirakul1, Ryan A Loyd2,3, Sukij Panpimanmas3,4, Likit Matrakool1,3,4, Taweesak Tongtawe3,4, Porntip Kompor5, Jun Norkaew5, Wasugree Chavengkun5, Jirawoot Kujapan5, Sukanya Polphimai5, Tanida Phatisena6, Thawatchai Eaksunti6, Poowadol Polsripadist7, Natnapa Padchasuwan8, Nathawut Kaewpitoon1,3,5

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%). O. viverrini infection rate was increased from year 2012 to 2015 particularly in Bua Yai and Mueang Yang. These re-examination 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 underestimate 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 (Jongsuksantikul and Insomboon, 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
The O. viverrini infection in Thailand was the first reported in 1955 (Sadan) and many strategies have been implemented to control the prevalence of O. viverrini infection. In 2012, 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 community provinces of Northeast (Sittihawthon et al., 2012). In 2009, 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 (100,000 populations. Eradicating of the fluke and identification of high-risk populations are urgently needed (Sripa et al., 2009). In addition, the distribution of O. viverrini infection in Nakhon Ratchasima province has been reported, the prevalence of survey in 2009 was 4.6% (Sittihawthon et al., 2012). A total of 1,168 stool samples were collected from different study sites of Nakhon Ratchasima province, and investigated for O. viverrini metacerariae. The infection rate was 12.3% (79/640), predominantly in Cyclocoelichthys armatus, C. repassus, Puntioploes protzmanni, Hampula macropleitou and Hampula dispar, respectively. The prevalence of O. viverrini metaceraria was found covered 78.1% of areas, predominantly in Sida and KhaKham Thale So (Kae Wittpon 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) Ban Mai Chaiyaphut, Phutthamonthon and Kho Muang of Buriram province, Lam Thamnanchai, Chum Phuang and Pratthai of Nakhon Ratchasima Province. This district is subdivided into 9 sub-districts, and 44 villages. This district is coverage areas 255.5 km² (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 study. Briefly, a 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-epithoresisitive 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 O. viverrini infection had fallen from 63.6% to 9.6%

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

Discussion

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

| Characteristic | 2012 | 2015 |
|---------------|------|------|
| Age 20-39     | 229/645 (51.5%) | 41/175% |
| 40-49         | 126/359 (48%)   | 41/175% |
| ≥50           | 29/8.17%        | 0      |
| Male          | 291/50%         | 60/24% |
| Female        | 154/50%         | 57/26% |
| District       |                  |       |
| Bua Yai       | 121/34(08%)     | 3/32%  |
| Chum Phuang   | 165/54(95%)     | 3/34%  |
| Mueang Yang   | 71/20%          | 2/28%  |
| Total         | 355/100%        | 8/22.5%|

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

| Characteristic | 2012 | 2015 |
|---------------|------|------|
| District       |      |      |
| Bua Yai       | 36/0 | 121(3.24%) |
| Chum Phuang   | 36/0 | 163(4.14%) |
| Mueang Yang   | 36/0 | 71(2.82%) |
| Total         | 108/2(11.85%) | 355/8(22.5%) |
The *O. viverrini* infection in Thailand was the first reported in 1955 (Sadun) and many strategies have been implemented. The full 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 northeastern Thailand (Sithithaworn et al., 2012). 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 13.6:17.6:2.6 per 100,000 populations. Eradication of the fluke and identification of high-risk populations are urgently needed (Sadun et al., 2008). In addition, the distribution of *O. viverrini* infection in Nakakhon Ratashima province has been reported. Analysis of prevalence in 2009 was 4.6% (Sithithaworn et al., 2012). A total of 1,168 stool samples were collected from 66,163 populations from 3 districts of Nakhon Ratchasima province, and investigated in this study. 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-Kato-Katz procedure and examined 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) cholicesicosistis: definitive diagnosed by medical doctor or related officers, (5) relative family with infected with *O. viverrini*. (Kaewpitoon et al., 2012). This study aimed to re-examine *O. viverrini* infection in 3 districts of Nakakhon Ratashima province using multi-stage sampling technique, between June and October 2015. This data is 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 Nakakhon Ratashima province, northeastern Thailand, between June and October 2015, included Bua Yai, Chum Phuang, and Chum Phuang district (Figure 1). Bua Yai is a district in the northern part of Nakakhon Ratashima, and neighboring districts are (from the north clockwise) Prathai, Mueang Yang and Lam Thamenchai of Nakakhon Ratashima Province, Lam Plai Mat of Buriram province, and Huai Thalaeng and Phimai of Nakhon Ratchasima. The district is subdivided into 9 sub-districts, and 130 villages. This district is coverage areas 540.6 km² (208.7 sq mi), and has 82,161 populations. Mueang Yang is a district in the northeastern part of Ratashima province, neighboring districts are (from 00, north clockwise) Ban Mai Chaiyayut, Phutthaisong and Kho Mueang of Buriram province, Lam Thamenchai, Chum Phuang and Prathai of Nakakhon Ratchasima Province. The district is subdivided into 4 sub-districts, and 44 villages. This district is coverage areas 255.5 km² (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 study. Each slide (Meireles et al., 2008). All preparations were preserved in 10% formalin for later confirmation, if needed. *O. viverrini* positive case was confirmed by 2 expert pathologists and confirmed under a high-power (40x) objective lens. The stained slides were examined by the expert parasitologists before a definitive diagnosis was made. 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. SPSS software version 12.0 was carried out using SPSS software version 12.0. The protocol study was approved by Suvarnaree University Ethical Review Committee, EC58-48.

**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 (3.3%) more than male (1.7%). 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 in age between 41-50 years old, and followed by 61-70 years old and 51-60 years old (1.54%). respectively. *O. viverrini* infection was found in the highest age in Mueang Yang district (2.82%), 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 stercoralis. 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 (0.1%) and Mueang Yang (2.78%) district in year 2015 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**

Opisthorchiasis is still a serious health problem in Nakakhon Ratchasima province. A high prevalence of *O. viverrini* infection was surveyed in 2009 and 2012, *O. viverrini* infection was decreased. *O. viverrini* infection was surveyed in 2009 and found that Nakakhon Ratashima province had 4.6% accumulation of 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 perpendicularly 4x10.1 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 the orifice to remove any 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 it was allowed to dry while 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 lens. The stained slides were examined by the expert parasitologists before a definitive diagnosis was made. 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. SPSS software version 12.0 was carried out using SPSS software version 12.0. The protocol study was approved by Suvarnaree University Ethical Review Committee, EC58-48.

**Table 1. Baseline characteristics of participants in 3 districts of Nakakhon Ratashima province, Thailand.**

| Characteristics | No. n (%) | No. of infection n(%) |
|-----------------|-----------|-----------------------|
| Gender          |           |                       |
| Male            | 229(64.51%)| 4(1.75%)              |
| Female          | 126(35.49%)| 2(1.54%)              |
| Age             |           |                       |
| 30-40           | 29(8.17%) | 2(2.48%)              |
| 41-50           | 89(25.07%)| 3(4.49%)              |
| 51-60           | 130(36.62%)| 2(1.54%)              |
| >60             | 98(27.41%)| 2(2.04%)              |
| District        |           |                       |
| Bua Yai         | 121(34.08%)| 3(2.48%)              |
| Chum Phuang     | 165(45.92%)| 3(1.84%)              |
| Mueang Yang     | 71(20%)   | 2(2.82%)              |
| Total           | 355(100%) | 8(2.25%)              |

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

| District        | 2012 No. | 2015 No. |
|-----------------|----------|----------|
| Bua Yai         | 36(0%)   | 121(3.24%)|
| Chum Phuang     | 36(1.72%)| 163(3.84%)|
| Mueang Yang     | 36(1.72%)| 71(2.28%)  |
| Total           | 108(2.15%)| 355(8.25%)|

**Figure 1. Map of Nakakhon Ratashima province, Northeastern Thailand; blue color lines are 3 districts of study areas including Bua Yai, Mueang Yang, and Chum Phuang.**

**Figure 2. Diagram of Participant Selection in 3 Districts of Nakakhon Ratashima Province, Thailand, Using Multi-stage Sampling Technique**

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

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(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 cholangiocarcinoma in infected participant and risk group is urgently concerned.

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