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

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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 Ratrasima 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-examination results indicate that opisthorchiasis is still problem in community of Nakhon Ratrasima 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 Ratrasima - 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* (Jongsuksaktikul 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 (Jongsuksaktikul and
The Opisthorchiasis infection in Nakhon Ratchasima province, Northeastern Thailand: A cross-sectional survey.

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The Opisthorchiasis infection in Thailand was first reported in 1955 (Sadak) and many strategies have been implemented to reduce the prevalence of Opisthorchiasis infection in Thailand. The recent report (Kaewpitoon et al., 2012) showed that Nakorn Ratchasima province has 13.67-16.2 per 100,000 populations. Eradication of the fluke and infection is necessary to reduce the risk of the adult and further transmission in livestock. Therefore, the objectives of this cross-sectional survey were to assess the rate of infection for Opisthorchiasis infection in Nakorn Ratchasima province, to identify risk areas for infection and to evaluate the current strategies implemented to reduce the prevalence of infection in Nakorn Ratchasima province.

Materials and Methods

A cross-sectional survey was a pilot project and conducted in 3 districts of Nakorn Ratchasima province, northeastern Thailand, between June and October 2015. Data was collected from 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 for O. viverrini and other known intestinal parasitic infection. A high score more than 5 points was selected, included 71, 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 that contained exactly 0.04 g or 40 µL of stool, at one corner of the slide. The other side of the slide was passed over an icebox and the sample was then transported in a nylon sieve 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.

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 aged 50 years old (4.94%), 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 stercoralis. 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. All data were transferred out using SPSS software version 12.0. The study protocol was approved by Suranaree University Ethical Review Committee, E58-48.

Discussion

Opisthorchiasis is still a serious health problem in Nakorn Ratchasima province. The 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 Nakorn Ratchasima province had 4.6% infection.
The O. viverrini infection in Thailand was the first reported in 1955 (Sadhan) and many strategies have been implemented to control it, including 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 (Sitithiwat, 2012). In addition, the high mortality rate of CCA was reported in the northeast areas where found frequently of O. viverrini infection (Sriya, 2008). 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 (Sriya, 2008). In 2012, the distribution of O. viverrini infection in Nakakon Ratchasima province has been reported, the prevalence of survey in 2009 was 4.6% (Sitithiwat, 2012). A total of 1,168 stool samples were obtained from 516 males and 652 females, aged 5-70 years. Stool examination showed that 2.25% 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 Nakakhon Ratchasima province, and investigated for O. viverrini metacercariae. The infection rate was 12.3% (79/640), predominantly in Cyclocheilichthys armatus, C. rapasan, Puntius protosynus, Hamphala macroleptota and Hamphala dispur, respectively. The prevalence of O. viverrini metacercia was found covered 78.1% of areas, predominantly in Sida and Khakham 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 Nakakon Ratchasima province using multi-stage sampling technique, between June and October 2015. This data is usefull 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 Nakakon 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 Nakakon Ratchasima, and neighboring districts are (from the north clockwise) Prathai, Mueang Yang and Lam Thamenchai of Nakakon Ratchasima Province, Lam Plai Mat of Buriram province, and Huai Thalaeng and Phimai of Nakakon Ratchasima again. 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 Nakakon Ratchasima province, neighboring districts are (from north, clockwise) Ban Mai Chaiyaut, Phutthaisong and Kho Mueang of Buriram province, Lam Thamenchai, Chum Phuang and Prathai of Nakakon Ratchasima Province. This 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 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- suspicion questionnaires containing the history with (1) epistoreschitsis; definitive diagnosed by medical doctor or related officers, (2) under-cooked fish consumption, (3) praziquantel used; given by medical doctor or related officers, (4) echellectyisis; definitive diagnosed by medical doctor or related officers, (5) relative family with cholangiocarcinoma, (6) naïve northeastern people, (7) agriculture, and (8) alcohol consumption. Population at risk was identified following 1+3+4+5+6+7+8, who had a high score more than 5 points was selected. After that, 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 4x10x 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 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 analysis was carried out using SPSS software version 12.0. The study protocol was approved by Suranaree University Ethics 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 (3.17%) more than male (1.75%). The majorities of O. viverrini infection were found in age group 50-60 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 highest in Mueang Yang district (2.82%), followed by Bua Yai (2.48%), and Chum Phuang (1.84%), respectively. Baseline characteristics infection was found the highest in Mueang Yang district (2.78%), respectively. Table 1. shows the characteristics of participants in 3 districts of Nakakon Ratchasima province, Thailand. Table 2. shows the characteristics of O. viverrini infection in 3 districts of Nakakon Ratchasima province between 2012 and 2015.

Discussion

Ophiothrix is still a serious health problem in Nakakon Ratchasima province, 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%) 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).
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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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