RESEARCH ARTICLE

# *Opisthorchis viverrini* Infection Among People in the Border Areas of Three Provinces, Northeast of Thailand

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## Abstract

***Opisthorchis viverrini* is still a serious problem in Northeastern and Northern Thailand. Active surveillance is required to determine updated data for further prevention and control planning. Therefore, this study aimed to examine the prevalence and risk factors for *O. viverrini* in three provinces, Northeastern Thailand. A cross-sectional survey was conducted during October 2015 to March 2016 at Kaeng Sanam Nang district of Nakhon Ratchasima province, Waeng Noi district of Khon Kaen province, and Khon Sawan district of Chaiyaphum province, Thailand. Stool samples were examined by using a modified Kato-Katz Thick smear technique. From a total of 978 participants screened, *O. viverrini* infection was found in 1.74%, the majority opf positive cases being male (6.62%), age group 51-60 years old (4.21%), educated at primary school (8.43%), occupied with agriculture (9.62%),having an income <4,000 baht per month (4.82%), and living in Khon Sawan district (8.43%). Participants had a high knowledge level (42.43%), good attitude level (34.76%), and fair level (38.04%). The present study indicates the *O. viverrini* infection rate is low, but elderly males with primary school education involved in agriculture are still frequently effected particularly in Khon Sawan district. Therefore, this risk group requires behavior modification and continued monitoring.**

**Keywords:** *Opisthorchis viverrini* - northeast Thailand - infection rate - risk factors - modified Kato Katz technique

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## Introduction

*Opisthorchis viverrini* remains a major public health problem in Thailand (Sithithaworn et al., 2012). *O. viverrini* is still found in the rural areas of Thailand particularly northeastern and northern region (Kaewpitoon et al., 2015). A nationwide survey in Thailand has been reported and found that the prevalent was 5.1%. The highest prevalent was found in the northeast (9.2%) and followed by the north region (5.2%) (Wongsaroj et al., 2014). This figure indicated that this province is still has a problem with *O. viverrini* particularly in the rural communities. The O.viverrini infection is associated with hepatobiliary diseases including hepatomegaly, cholangitis, cholecystitis, and gallstones Harinasuta et al., 1960; Thamavit et al., 1978; Harinasuta et al., 1984). Furthermore, epidemiological studies have determined that the incidence of cholangiocarcinoma (CCA) correlates strongly with the prevalence of *O. viverrini* infection (Parkin et al., 1993; Jongsuksuntigul and Imsomboon, 2003; Parkin, 2006; Sripa et al., 2007; Sripa et al., 2008; Shin et al., 2010). Recently, it has been classified as Type 1 carcinogens by the International Agency for Research on Cancer, World Health Organization (WHO) (IARC, 1994). In addition, a problem regarding opisthorchiasis and CCA in Thailand indicates that costs at least $120 million annually in both medical care and lost wages (Andrews et al., 2008).

Khon Kaen, Chaiyaphum, and Nakhon Ratchasima province are located in the northeastern region where are neighbor border areas including three district; Kaeng Sanam Nang, Khon Sawan, and Waeng Noi district. *O. viverrini* infection has been reported and found that Khon Kaen and Chaiyaphum province were high infection rate (Wongsaroj et al., 2014). Moreover, CCA has been reported the incident rate was 20.66-44.31. Meanwhile, liver cancer and cholangiocarcinoma in Nakhon Ratchasima province has been reported at 13.67-16.20 per 100,000 populations w (Sripa et al., 2008). Therefore, eradication of the fluke populations is urgently needed, this study aimed to examine the *O. viverrini* infection among three districts where are located along border areas of Khon Kaen, Chaiyaphum, and Nakhon Ratchasima province, by using modified Kato Katz thick smear technique. *O. viverrini* infection rate is useful indicate the current status at the community level.

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## Materials and Methods

A cross-sectional survey was conducted during October 2015 to March 2016 at Kaeng Sanam Nang district, Nakhon Ratchasima province, Khon Sawan district of Chaiyaphum province, and Waeng Noi district of Khon Kaen province, northeast of Thailand. These 3 neighbor districts are located in the provincial border areas. The study protocol was approved by Suranaree University Ethical Review Committee, EC58-63. Participants were calculated following Krejcie and Morgan (1970) and then randomly sampling from each district. Participant had completed the predesigned questionnaire with the validity and reliability of knowledge (Kruder-Richardon-20) = 0.80, attitude and practice (Cronbach's alpha coefficient) = 0.82 and 0.79, respectively. KAP questionnaire was utilized to collect the data from all participants. The KAP questionnaire was comprised 5 domain containing (1) demographic data; gender, age, education, agriculture, and income, (2) knowledge; 20 questions, (3) attitude; 20 questions, and (5) practice; 20 questions. Each questionnaire was analyzed and interpreted for their parts. Evaluation of knowledge level was calculated and analyzed according to Bloom (1971), answer correct=1, incorrect=0, and interpreted to high level; 16-20 points, moderate level; 12-15 points, 0-11 points; low level. Evaluation of attitude

**Table 1. Demographic data and *O. viverrini* Infection among People in the Border Areas of three Province, Northeast of Thailand**

| Demographic data | | Number of Participant | | *O. viverrini* positive | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| Gender | | | | | |
| | Male | 392 | 40.08 | 11 | 6.62 |
| | Female | 586 | 59.92 | 6 | 3.61 |
| Age | | | | | |
| | >40 year | 80 | 8.18 | 2 | 1.21 |
| | 41-50 year | 331 | 33.84 | 4 | 2.40 |
| | 51-60 year | 357 | 36.50 | 7 | 4.21 |
| | <61 year | 210 | 21.47 | 4 | 2.40 |
| District | | | | | |
| | Kaeng Sanam Nang | 319 | 32.62 | 3 | 1.80 |
| | Khon Sawan | 377 | 38.55 | 14 | 8.43 |
| | Waeng Noi | 282 | 28.83 | 0 | 0.00 |
| Education | | | | | |
| | Uneducated | 2 | 0.20 | 0 | 0.00 |
| | Primary School | 617 | 63.09 | 14 | 8.43 |
| | High Shool | 334 | 34.15 | 3 | 1.80 |
| | Diploma | 6 | 0.61 | 0 | 0.00 |
| | Undergraduate | 19 | 1.94 | 0 | 0.00 |
| Occupation | | | | | |
| | Agriculturist | 865 | 88.45 | 16 | 9.62 |
| | No agriculturist | 53 | 5.42 | 0 | 0.00 |
| | Contractors | 60 | 6.13 | 1 | 0.60 |
| Income(Thai Baht) | | | | | |
| | <4,000 | 419 | 42.84 | 8 | 4.82 |
| | 4,000-8,000 | 260 | 26.58 | 6 | 3.61 |
| | <8,000 | 299 | 30.57 | 3 | 1.80 |
| Total | | 978 | 100 | 17 | 1.74 |

**Table 2. Behavior Regarding *O. viverrini* Infection Among people in the Border Areas of three Province, Northeast of Thailand**

| Behavior | | Number of Participant | | *O. viverrini* negative | | *O. viverrini* positive | |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % |
| Knowledge | | 978 | 100.00 | 961 | 98.26 | 17 | 1.74 |
| | Low | 391 | 39.98 | 382 | 97.70 | 9 | 2.30 |
| | Moderate | 172 | 17.59 | 167 | 97.09 | 5 | 2.91 |
| | High | 415 | 42.43 | 412 | 99.28 | 3 | 0.72 |
| | | $X^2$=0.100, p-value=4.607 | | | | | |
| Attitude | | 978 | 100.00 | 961 | 98.26 | 17 | 1.74 |
| | Low | 270 | 27.61 | 264 | 97.78 | 6 | 2.22 |
| | Moderate | 368 | 37.63 | 363 | 98.64 | 5 | 1.36 |
| | High | 340 | 34.76 | 334 | 98.24 | 6 | 1.76 |
| | | $X^2$=0.711, p-value=0.682 | | | | | |
| Practice | | 978 | 100.00 | 961 | 98.26 | 17 | 1.74 |
| | Low | 264 | 26.99 | 257 | 97.35 | 7 | 2.65 |
| | Moderate | 372 | 38.04 | 365 | 98.12 | 7 | 1.88 |
| | High | 342 | 34.97 | 339 | 99.12 | 3 | 0.88 |
| | | $X^2$=0.244, p-value=2.819 | | | | | |

level was calculated and analyzed according to Likert (1932) with 3 choice (agree, not sure, dis-agree): positive question=3,2,1, negative question=1,2,3, and interpreted to good level; 48-60 points, fair level; 36-45 points, and poor level; 0-35 points. Evaluation of practical level was calculated and analyzed according to Best (1977) with 3 choice (frequently, sometimes, never): positive question=3,2,1, negative question=1,2,3, and interpreted to good level; 48-60 points, fair level; 36-45 points, and poor level; 0-35 points.

Stools were collected and kept in labeled plastic bags and transported in an icebox to the laboratory at the Parasitic Disease Research Unit, Institute of Medicine, Suranaree University of Technology, Thailand, within a day after collection. Stool specimens were examined for *O. viverrini* egg by the modified Kato Katz thick smear procedures according to the method of WHO (1991). Briefly, 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. In order to eliminate fiber or seed the technique modified by pressing a 105-mesh stainless steel grid onto the sample and then filtered. Stool was 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. *O. viverrini* positive case was confirmed by 2 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.

## Results

A total of 978 populations was completed a predesigned questionnaires and stool examination in this study. The majorities were female (59.92%), age group 51-60 years old (36.50%), educated with primary school (63.09%), agriculture (88.45%), income < 4,000 baht per month (42.84%), and Khon Sawan district (38.55%), respectively. *O. viverrini* infection was 1.74%, the majorities of infection were male (6.62%), age group 51-60 years old (4.21%), educated with primary school (8.43%), agriculture (9.62%), income <4,000 baht per month (4.82%),, and Khon Sawan district (8.43%) (Table 1). Behavior was completed analyzed and found that the majorities of participants had a high knowledge level (42.43%), good attitude level (34.76%), and fair level (38.04%) (Table 2). The association between demographic, behavior, and *O. viverrini* infection was analyzed and found that demographic and behavior data were not statistically significant associated to *O. viverrini* infection.

## Discussion

*O. viverrini* has been classified as a group 1 biological carcinogen and it is associated to chonlagiocarcinoma (CCA) (IARC, 1994). The first human cases of *O. viverrini* infection were reported in Thailand 100 years ago. Presently, based on nationwide surveyed in 2014, approximately 3.3 million people were infected with *O. viverrini*. The highest of prevalent was found in the northeast (9.2%) and followed by the north region (5.2%) (Wongsaroj et al., 2004). Active surveillance for the five years period from 2010 and 2015, community-based surveys were demonstrated a high prevalence over 20%, and the highest was 45.7% (Kaewpitoon et al., 2015). The prevalence of *O. viverrini* infection remains high in various parts of the country, especially in northeast Thailand and particularly in wetland rural areas where a large proportion of the community work in agriculture and continue the traditional practice of eating raw or undercooked cyprinoid fish products. The national control program seems to have had little impact in many of these areas, and it has been difficult to make precise assessments of the overall effectiveness of the program (Sripa et al., 2007; Sitthithaworn et al., 2012). Here we examined the *O. viverrini* infection neighbor district of three province located in northeastern province by using modified Kato Katz thick smear. The result reveals that infection rate of this study (1.74%) was lower than national survey in 2014 (5.10%) by Wongsaroj et al. Differences in the prevalence of infection presumably reflect variations in environmental conditions and social behavior (Wongsaroj et al., 2014). The variability between different geographic regions is shown along with seasonal variations that might serve as an explanation for this discrepancy (Sitthithaworn et al., 1997). The variation even within the high risk regions might lack of culturally sensitive and educationally informed information concerning "raw attitudes" in eating behavior of the people in the Lower Mekong Basin (Grundy-Warr,et al., 2012). Governmental control programs may be partially successful during active campaigns but this is not sustainable. Control strategies, therefore, should be designed to accommodate a more community-oriented approach and not a top down policy. Ecosystem health or EcoHealth is one of the approaches that may be ideal for liver fluke control, since the disease is a complex problem involving not only humans but several intermediate and reservoir hosts, different environments, government and non- government sectors including communities and individual villages (Sitthithaworn et al., 2012). The highest of infection was found in Khon Sawan district of Chaiyaphum province with 8.43%, and followed by Kang Sanam Nang district (1.80%). This data shows that an infection rate of Khon Sawan district was higher than the prevalent of national survey. Epidemiological data has been reported that the incident rate of CCA was 20.66-44.31 in Chaiyaphum (Sripa et al., 2008). Surprisingly, infection was not found in Waeng Noi district of Khon Kaen province, this is may useful of continue prevention and control campaign in Khon Kaen province for along time after they have been reported a highest of CCA incidence. However, a limitation of the diagnostic efficiency of Kato Katz thick smear is that its specificity and difficult to standardize, can be reduced (Lovis et al., 2009; Sayasone et al., 2015). In addition,

*O. viverrini* infection is fluctuated in the epidemic areas mainly we reported the re-examination of *O. viverrini* in Nakhon Ratchasima province and found that prevalence of *O. viverrini* infection was 2.25%. *O. viverrini* infection rate was increased from year 2012 to 2015 particularly in Bua Yai and Mueang Yang district (Kaewpitoon et al., 2016).

*O. viverrini* infection is found frequently in male, age group 51-60 years old, educated with primary school, agriculture, and income <4,000 baht per month, this result is similarly to previous that is a cross-sectional survey using multistage sampling was conducted from the rural communities of Surin province, Thailand, during September 2013 to July 2014. 6.47% of participants were infected with *O. viverrini*. The rate was slightly higher in males (6.61%) than females (6.32%). High frequencies were found in the age groups 61-70 (19.4%) and 71-80 years (19.4%), those involved in agriculture (10.5%), and in primary school (10.3%) (Kaewpitoon et al., 2015). Furthermore, a community-based cross-sectional survey was conducted among 333 elderly in 17 districts of Surin province, during one year period from January to December 2011. Overall intestinal parasitic infection was 16.2%, predominantly in *O. viverrini* (9.91%) and followed by Strongyloides stercolaris (4.80%) and hookworm (1.50%), respectively. The *O. viverrini* infection was found higher in males (13.8%) than females (7.83%), and frequently in elderly 60-70 year old with 14.2%. recently, data derived from our study demonstrated that there are still groups predisposed to high infection rates, possibly because of higher rates of exposure through work activities and consumption of raw fish. However, demographic data was no statistically significant associated to *O. viverrini* infection. This is different to previous study in epidemic areas surveyed of Surin found that Chi-square testing indicated that age (61-70 and 71-80 year old), education (primary school) and occupation (agriculture), were significantly associated with *O. viverrini* infection (p-value<0.05). In addition, Chi-square testing indicated that education and occupation were significantly associated with infection (P value = 0.02). It possibly because of different areas and culture may due to various risk factors.

Behavior was completed analyzed and found that the majorities of participants had a high knowledge (42.43%), good attitude (34.76%), and good practice (38.04%). These results demonstrated that some rural villagers are still a misunderstood and misbelief to liver fluke. Inadequate knowledge, misbeliefs, and social and cultural mores were important factors leading to the maintenance of risk behaviors. Moreover, unhygienic defecation and insufficient diagnosis and treatment were found to facilitate *O. viverrini* transmission. Consumption of chopped raw fish salad and Koi pla were independently associated with *O. viverrini* infection (Suwannahitatorn et al., 2013). Therefore, health education is need required in the moderate level, meanwhile health behavior modification is required to whom that have a fair or poor attitude and practice, and low knowledge regarding liver fluke prevention and control.

The present study indicates the *O. viverrini* infection rate was row, however male, elderly age, primary school agriculture are still found frequently particularly in Khon Sawan district. Therefore, risk group is required behavior modified and continue monitored.

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