

RESEARCH ARTICLE

A Large Scale Study of the Epidemiology and Risk Factors for the Carcinogenic Liver Fluke *Opisthorchis viverrini* in Udon Thani Province, Thailand

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Abstract

Opisthorchis viverrini infection and cholangiocarcinoma are serious problems in South East Asia. This study aimed to find the prevalence of opisthorchiasis in various hosts in Udon Thani Province. Total fecal samples were collected from 14,766 participants. The epidemiological data collected and analysed included prevalence and intensity of infection. Odds ratios (OR) were calculated to determine the associations between cross sectional data and to predict possible risk factors. The prevalence of *O. viverrini* infection in Udon Thani Province averaged 15.3% (eggs per gram (epg.) = 48.9 and range; 12-1,320), with differences between villages (range; 3.8%-79.8%). An age-dependence for infection was observed to increase from ages 25 to 50 years and then decrease for older participants. A univariate analysis identified risk parameters including age ($p = 0.040$; OR = 3.9 (95% CI = 1.2-7.5)), education ($p < 0.0001$; OR = 7.3 (95% CI = 1.8-21.6)) and eating habits ($p = 0.032$; OR = 1.6 (95% CI = 0.5-3.7)). Interestingly, most participants were not aware of treatments such as praziquantel ($p < 0.0001$; OR = 3.5 (95% CI = 1.4-11.6)), had no history of parasitic treatment ($p = 0.486$; OR = 1.5 (95% CI = 0.5-3.5)) and had eaten raw fish ($p = 0.04$; OR = 7.4 (95% CI = 1.5-18.6)). Liver fluke infection in dogs (18.1%, epg. = 44.7, range; 32-96) was significantly higher than in cats (11.0%, epg. = 117.8, range; 44-372) ($p < 0.05$). A positive association between *O. viverrini* infection in dogs and their owners was found. In addition, cyprinid fish dominantly infected by metacercaria including *Henicorhynchus siamensis* (27.7%), *Cyclocheilichthys repasson* (21.9%), *Hampala dispar* (14.1%), and *Barbonymus gonionotus* (6.9%). This study provides basic information required for the development of future effective and sustainable strategies to reduce infection rates, mainly by providing health education and encouraging behavioural changes.

Keywords: *Opisthorchis viverrini*- epidemiology- risk factors

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Introduction

Chronic *Opisthorchis viverrini* infection is a critical risk factor for hepatobiliary diseases and cholangiocarcinoma (CCA), which are health problems across South-East Asia, including Thailand, Laos People's Democratic Republic, Cambodia and central Vietnam (Sripa et al., 2007). In this endemic area at least 6 million are estimated to be infected and without diagnosis and treatment risk progression to CCA (Jongsuksuntigul and Imsomboon, 2003). In addition, people traditionally eat fermented fishes containing high concentrations of nitrosamines which are potent human carcinogens (Thamavit et al., 1993). Despite recent reports indicating that highly effective antiparasitic treatments are frequently used, the prevalence and reinfection rates for liver fluke are still high (Saengsawang et al., 2017).

O. viverrini infection in definitive and reservoir hosts was occurring as a result of consuming raw or uncooked fish which contain the infective metacercaria (Sripa et al., 2007). The parasite migrates to the intrahepatic bile ducts via the common bile duct and produces eggs that are excreted in the feces after approximately 30 days (Harinasuta and Harinasuta, 1984). Many reports have shown that the prevalence of *O. viverrini* in North-East Thailand has gradually decreased over thirty years from 34.6% to 10-15% depending on the area sampled (Jongsuksuntigul and Imsomboon, 2003), however the infection percentage has gone no lower than 10% in the endemic areas (Kaewpitoon et al., 2015). This may be due to the control strategies of recent decades being concentrated only on human infection. However, it is well known that the *O. viverrini* life cycle can also involve domestic reservoir hosts, such as dogs and cats

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(Sithithaworn and Haswell-Elkins, 2003). Despite, the human opisthorchiasis was treated but the food-borne parasitic zoonosis is a complete epidemiological cycle by facilitating return to human, which is still certain this phenomenon in endemic countries.

The critical risk factors for *O. viverrini* infection are mainly associated with eating raw or uncooked fish in many traditional fish dishes. There is evidence that 25 - 28 % of cyprinid fish were infected in the 13 provinces of the North-East region surveyed in the last decade. However, the rates of metacercaria infection was less and steady at about 12.4% in Nakhonratchasima Province, elsewhere in Thailand (Kaewpitoon et al., 2012a), 14.5% in 3 provinces of Lao PDR (Rim et al., 2013), 1.9% in Vietnam (Thu et al., 2007) and 2.1% in Cambodia (Touch et al., 2009). Species diversity and an abundance of cyprinid fishes in the North-East of Thailand and advantageous environmental conditions in certain seasons support the host-parasite population (Donthaisong et al., 2016). A previous survey revealed a seasonal variation for *O. viverrini* metacercaria infection in cyprinid fish with a peak in the dry season which may further increase the possibility of transmission (Sithithaworn et al., 1997). In addition, differences in education, social practices, economy and life styles amongst communities change the patterns of risk to eat inadequately cooked fish (Sripa et al., 2017). The risk factors in each area also need to be identified.

In Udon Thani Province, the prevalence of *O. viverrini* was reported in 2003 at 19% and the highest CCA ratio (44 : 100,000) was reported in the North-East (Sripa et al., 2003). However, a lack of epidemiological data has not allowed the situation in the last decade to be clarified. In addition, the risk factor and complete epidemiological data for existing parasitic cycles in intermediated and reservoir hosts have never been simultaneously determined in each endemic area. Therefore, the increased risk of opisthorchiasis-associated CCA still remains as a result of imprecise prevention and control strategies.

This study aimed to perform a large scale integrated study of the prevalence and intensity of *O. viverrini* infection in residents and reservoir hosts including domestic dogs and cats and at the infective stage (metacercaria) in cyprinid fishes in Udon Thani Province, an upper part of North-East, Thailand. Samples were collected from 176 villages and the number of eggs per gram of feces (epg.) was measured by a modified formalin ethyl-acetate concentration technique. The prevalence and density of metacercaria were determined monthly by conventional 0.25% pepsin digestion.

Materials and Methods

Study area and fecal sample collection

The epidemiology of opisthorchiasis in humans and reservoir hosts and the prevalence and density of metacercariae in cyprinid fish was studied as part of an outreach sustainable prevention and control strategy initiated by the Faculty of Science, Udon Thani Rajabhat University. Sample collection was conducted between October 2016 and July 2017 in 16 districts of Udon Thani

Province along the Huay Luang and Song Kram rivers, the Nong Han wet lands and the Nam Pan reservoir. Fecal samples were collected from 14,766 participants living in 176 villages of which 5,659 were males and 9,907 were females, with an age range spanning 25-76 years. The study protocol was approved by the Human Ethics Committee of Udon Thani Provincial Health Office, Ministry of Public Health, Thailand. Letters explaining the aims of the study and the strategies to be followed were sent to village heads, community leaders and to individual households. Visits were made to each village to explain the benefits of the survey and the national program. Risk factors for liver fluke infection were assessed by using questionnaires to gather information about the age, sex, occupation, knowledge of parasitic treatments and eating behaviors of the participants.

Fecal samples from domestic dogs (341) and cats (118) were collected from within 28 villages along the Huay Luang river basin. Only stools from domestic dogs and cats which had been confined to the house of their owners were collected thus allowing the fecal sample results to be correlated with the data obtained from the human occupants of the same house. The Animal Ethics Committee of Udon Thani Rajabhat University also approved this part of the study.

Humans and host reservoirs that were found to be parasitized by all species were treated with appropriate parasitic drugs and included in the active prevention and control program as part of the research strategy of the Faculty of Science, Udon Thani Rajabhat University.

A modified formalin ethyl-acetate concentration technique

A quantitative modified formalin ethyl-acetate concentration technique was used with slight modification. One gram of feces was fixed in 10% formalin and filtered through 2 layers of gauze after which the suspension was centrifuged at 2,500 rpm for 3 minutes. The pellet was re-suspended with 10 ml of 10% formalin and 3 ml of ethyl-acetate and again centrifuged at 2,500 rpm for 3 minutes. The floating liquid lipid in the supernatant was removed by tearing the inside of the tube. To the sediment was added 1 ml of 10% formalin and the number of eggs per gram of feces due to liver fluke infection was measured and confirmed by two experienced researchers.

Isolation of metacercariae from cyprinid fish

Cyprinid fish have traditionally been caught by villagers from wet lands, rivers, dams and reservoirs in Udon Thani Province. This study was conducted monthly between August 2016 and July 2017. *O. viverrini* metacercariae were isolated from naturally infected fish by pepsin digestion as described previously (Pinlaor et al., 2013). Briefly, the individual fish were minced by electric blender and digested with a digestive solution containing 0.25% pepsin and 1.5% HCl in 0.85 % NaCl followed by incubation at 37 °C for 2 hours. The digested fish were filtered sequentially by 4 metal sieves having 2100, 350 and 250 µm apertures. Filtered fish pellets were sedimented several times with normal saline in a sediment jar until the supernatant was clear. *O. viverrini* metacercariae in the sediment were identified under a

dissecting microscope, allowing the percentage infection and the density of metacercariae per gram of infected fish to be measured.

Statistical analyses

Data are presented as average infection percentages, intensity of infection including average epg., and the average metacercaria/gram of infected fish. One-way ANOVA was used to compare the intensities of epg. in feces and the infective rates for metacercariae in different kinds of fish. Student's t-test was used to make a parametric comparison by gender. Pearson's correlation coefficient was used to analyze correlations within the parametric data. Odds ratios (OR) were calculated to determine the association between cross sectional data to predict possible risk factors. Statistical analyses were performed using SPSS version 11.5 and p values less than

0.05 were considered statistically significant.

The administrative boundaries of Thailand and Udon Thani Province were downloaded from www.diva-gis.org and became the base layer for creating opisthorchiasis prevalence maps using the ArcGIS mapping software (ESRI, Redlands, CA). The spatial distribution of human *O. viverrini* infection in Udon Thani at the district level is shown in Figure 1.

Results

Prevalence and intensity of O. viverrini in humans

The measurement of prevalence and density of *O. viverrini* infection in humans were performed as part of a sustainable prevention and control strategy implemented within the community by the Faculty of Science, Udon Thani Rajabhat University and a total of

Table 1. Characteristics and Prevalence of *O. viverrini* in Participants

Characteristics	No. Samples	No. of infections	Prevalence (95% CI)	Average epg. (range)
Gender	9,907	1,294	13.0% (12.8-14.2)	22.6 (12-474)
Female	5,659	971	17.1% (14.8-19.4)	64.1 (19-1,320) ^a
Male				
Age				
25 - 30 yrs	668	87	13.0 % (9.8-16.4)	63.1 (12-374)
31 - 40 yrs	3,472	516	14.8% (13.1-16.8)	113.7 (18-228)
41 - 50 yrs	4,548	977 ^a	21.4% (19.7-22.2)	231.8 (45-1,320) ^a
51 - 60 yrs	2,781	429	15.4% (14.4-16.2)	23.5 (32-562)
>60 yrs	3,295	256	7.7% (5.8-8.3)	41.2 (41-735)
Occupations				
Agriculture	13,013	1,985	15.2% (14.9-15.8)	93.4 (12-653)
Fisherman	472	156	33.0% (26.4-37.1)	425.5 (18-1,320) ^a
Government	785	83	10.5% (7.0-13.2)	124.1 (56-327)
Others	496	41	8.2% (6.4-12.8)	87.0 (44-187)
Districts				
Muang -Down town	1,106	42	3.8% (1.4-4.3)	74.2 (35-164)
Muang - Urban	4,756	1,087 ^a	22.8% (18.4-28.1)	124.6 (18-1,320) ^a
Sri Tart	1,584	226	14.2% (12.5-16.3)	57.2 (63-324)
Nong Wua Sor	1,509	131	8.6% (6.3-9.5)	84.5 (37-768)
Nong Han	906	128	14.1% (12.5-17.3)	157.2 (39-452) ^a
Non Sa-ard	940	140	14.9% (12.0-17.3)	85 (45-355)
Tung Fon	491	62	12.6% (11.3-15.7)	53 (29-153)
Pi Bun Rak	632	110	17.4% (13.7-19.5)	183.5 (32-1,195) ^a
Phen	435	66	15.2% (13.9-16.5)	64 (43-461)
Khum Phavapi	754	63	8.3% (7.3-10.8)	79 (23-198)
Sang Kom	650	104	16.0% (15.2-17.7)	185.2 (46-687) ^a
Ban Dung	345	39	11.3% (8.3-12.1)	97 (38-663)
Na Yung	283	17	6.0% (4.2-6.9)	33 (26-157)
Ku Kaew	125	16	12.8% (11.8-14.4)	64 (59-187)
Wang Sam Mor	109	18	16.5% (14.7-18.1)	79 (36-297)
Kud Chab	78	12	15.3% (11.2-17.0)	154 (12-284)
Ban Puae	63	4	6.3% (4.4-7.2)	124 (56-426)
Total	14,766	2,265	15.3% (13.6-17.7)	48.9 (12-1,320)

a, significantly statistic at p<0.05.

Table 2. Prevalence and Density of *O. viverrini* in Reservoir Hosts and Humans

Host	No. of Samples	No. of infections	Prevalence (95% CI)	Average epg. (range in each village)
Dogs	341	62	18.1% a (15.7-22.7)	44.7 (32-96)
Cats	118	13	11.0% (8.6-12.8)	117.8 (44-372) ^a
Humans	14,766	2,265	15.3% (13.6-17.7)	48.9 (12-1,320)
Total/Average	15,225	2,340	15.3% (13.4-17.8)	50.1 (12-1,320)

a, statistical significance set at p<0.05

Table 3. Risk Factors for *O. viverrini* Infection in Udon Thani Province

Factors	No. of questionnaires (Total 2,271)	Infected (%)	P value	Crude odds ratio (95%CI)
Gender				
Male	912	118 (12.9)	0.074	1
Female	1,359	209 (15.3)		1.9 (0.8-14.3)
Age				
< 40 years	784	94 (11.9)	0.040	1
> 40 years	1,487	233 (15.6)		3.9 (1.2-7.5)
Education				
Higher than secondary school	749	72 (9.6)	0.000	1
Secondary school and under	1,522	255 (16.7)		7.3 (1.8-21.6)
Life style and food selection habits				
For nutritional value	774	129 (16.6)	0.032	1
Choice by tradition	1,497	170 (13.2)		1.6 (0.5-3.7)
Know of praziquantel				
Yes	741	76 (10.2)	0.000	1
No	1,530	251 (16.4)		3.5 (1.4-11.6)
History of parasitic treatment				
Ever	949	142 (14.9)	0.486	1
Never	1,322	185 (13.9)		1.5 (0.5-3.5)
Eaten raw fish in the last year				
Never	1,459	85 (5.8)	0.004	1
Often	812	242 (29.8)		7.4 (1.5-18.6)

14,766 participants were included in the study (Table 1). The prevalence of *O. viverrini* infection in Udon Thani Province was 15.3% (2,265 infected). The levels of infection were different in each village, with the highest rate being 79.8% (range; 3.8%-79.8%). There was no statistically significant difference between the percentage levels of *O. viverrini* infection in males (17.1%) and in females (13.0%). However, the average density differences were statistically different (64.1 epg. in males compared to 22.6 epg. in females). An age-dependence for *O. viverrini* infection was found and reached a peak within the 41-50 year age range (21.4%). The majority

of positive cases had been educated at secondary school (38.3%) and their most common occupations was fishing (33.0%). The highest prevalence (22.8%) and density (124.6 epg., range 18-1,320) was found in rural areas of the Muang District (p<0.05).

Prevalence of O. viverrini infection around river basins

The study covered 16 districts of Udon Thani Province, including the Huay Luang River, the Song Kram River, the Nong Han Wetland and the Nam Pan Reservoir. The highest incidences of infection in Udon Thani were found near water resources, especially the

Table 4. Prevalence and Density of *O. viverrini* Metacercariae in Cyprinid Fish

Host	No. of Samples	No. of infections	Prevalence	Average metacercaria/gram of infected fish (range)	Average metacercaria/infected fish (range)
<i>Henicorhynchus siamensis</i>	671	186	27.7% ^a	2.8 (1-36) ^a	17.6 (4-125) ^a
<i>Cyclocheilichthys repasson</i>	748	164	21.90%	1.4 (1-24) ^a	14.7 (5-137) ^a
<i>Hampala dispar</i>	254	36	14.10%	0.2 (1-16)	7.9 (4-162)
<i>Barbonymus gonionotus</i>	158	11	6.90%	ND*	ND*
Total/Average	1,831	397	21.60%	1.5 (1-36)	15.7 (4-162)

a, significant p < 0.05; ND, Not determined

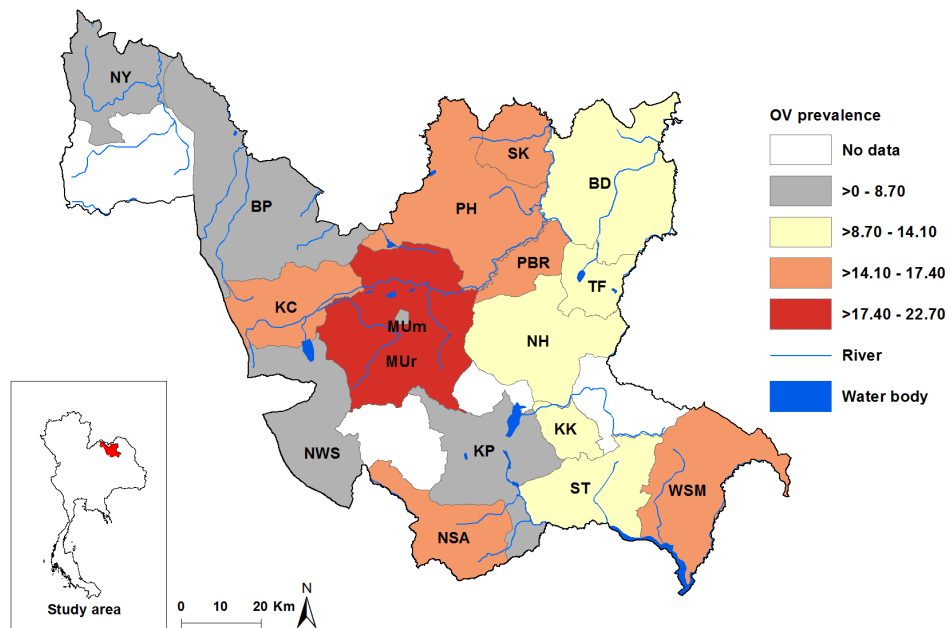


Figure 1. Prevalence of *O. viverrini* Infection in Udon Thani Province. The distribution of infection is shown for each district. Symbol of district is as follows; Mum, Muang –Downtown; Mur, Muang urban area; ST, Sri Tart; NWS, Nong Wua Sor; NH, Nong Han; NSA, Non Sa-ard; TF, Tung Fon; PBR, Pi Bun Rak; PH, Phen; KP, Khum Phavapi; SK, Sang Kom; BD, Ban Dung; NY, Na Yung; KK, Ku Kaew; WSM, Wang Sam Mor; KC, Kud Chab; BP, Ban Puae.

Huay Luang River in the Muang District (22.8%; 124.6 epg., range; 18-1,320), the Nong Han Wetland in the Nong Han District (14.1%; 157.2 epg., range; 39-452), the Pi Bun Rak District (17.4%; 183.5 epg., range; 32-1,195), the Nam Pan Reservoir in the Sang Kom District (16.0%; 185.2 epg., range; 46-687) and in the Song Kram River in the Tung Fon District (12.6%; 53 epg., range; 29-153). Locations away from rivers and at higher altitude showed lower levels of infection (Figure 1).

Prevalence and density of *O. viverrini* in reservoir hosts

To clarify the critical role of dogs and cats in promoting liver fluke infection in Udon Thani Province, the prevalence of *O. viverrini* infection in reservoir hosts is shown in Table 2. The rates of liver fluke infection in dogs (18.1%) was significantly higher than in cats (11.0%) ($p < 0.05$). The average epg. in feces in dogs were found to be 44.7 epg. (range; 32-96), in cats 117.8 epg. (range; 44-372) and in humans 48.9 epg. (range; 12-1,320) with the difference between dogs on the one hand and humans and cats on the other significant at the $p < 0.05$ level. The average infection rates across the three hosts was 2,340 or 15.3%. All of the infected hosts were treated by praziquantel and included in the active prevention and control program being undertaken as a research initiative of the Faculty of Science, Udon Thani Rajabhat University.

Association between *O. viverrini* infection in dogs and their owners

The association between liver fluke infection in dogs and their owners in the Huay Luang River area was analyzed by Pearson's correlation coefficient for parametric data. Of the 72 houses including infected

houses, a statistically significant correlation between *O. viverrini* infection in dogs and humans was found ($p < 0.05$, $r = 0.734$). Interestingly, in the 34 houses in which humans and dogs were both infected the epg. in humans (64.6, range; 48-749) was significantly correlated with the epg. in their domestic dogs (61.9, range; 52-644) ($p < 0.05$, $r = 0.641$).

Risk factors of *O. viverrini* infection

To determine which variables could be used to predict the risk of parasitic infection, we calculated odd ratios from 2,271 collected questionnaires (15.3% of collected fecal samples) as shown in Table 3. In a univariate model, the risk factors significantly related to *O. viverrini* infection included age ($p = 0.040$; OR = 3.9), education ($p < 0.0001$; OR = 7.3) and habits related to food selection and cooking ($p = 0.032$; OR = 1.6). Interestingly, there was a statistically significant relationship between being an aware that praziquantel was an effective treatment for liver fluke infection and rates of infection ($p < 0.0001$; OR = 3.5). Any history of parasitic treatment was not statistically significant for *O. viverrini* infection ($p = 0.486$; OR = 1.5). As an observation, many participants were familiar with the anthelmintic drug albendazole. The behavior to eat raw fish was a good predictor of *O. viverrini* infection in the areas studied ($p = 0.04$; OR = 7.4). Data related to other factors was also collected such as occupation ($p = 0.26$; OR = 1.4), alcohol consumption ($p = 0.06$; OR = 1.7), smoking ($p = 0.09$; OR = 1.2) and income level ($p = 0.32$; OR = 1.5) but these factors were not statistically significantly related to levels of *O. viverrini* infection (data not shown).

Prevalence and density of O. viverrini metacercaria in cyprinid fish

The prevalence and density of the infective stage of *O. viverrini* was assessed by conventional pepsin digestion. Cyprinid fish were collected from natural water resources in Udon Thani Province and the proportions of the various species caught are shown in Table 4. The highest significantly significant difference prevalence amongst the four species of fish was *Henicorhynchus siamensis* ($p < 0.05$) (in Thai, Pla Kao Na (27.7%, $n = 671$)). In descending order of prevalence were *Cyclocheilichthys repasson* (Pla Sai Ton (21.9%, $n = 748$)), *Hampala dispar* (Pla Kasub Jud (14.1%, $n = 254$)) and *Barbonymus gonionotus* (Pla Ta-Pein (6.9%, $n = 158$)). We found that the average density of metacercaria in each infected fish species ranged from 4-162 but were statistically significantly higher in *Henicorhynchus siamensis* and *Cyclocheilichthys repasson* compared to *Hampala dispar* and *Barbonymus gonionotus* ($p < 0.05$).

The monthly prevalences of metacercaria in the endemic areas studied has been first measured by this study. The prevalence and density of metacercariae infection in *H. siamensis* was significantly higher and reached a peak during the December – February period (18%, range; 22-162 metacercariae/infected fish, $p < 0.05$), whereas levels of infection diminished during the August – October period (6%-1%, range; 4-94 metacercariae/infected fish) indicating that the prevalence and of metacercaria was associated with the raining and dry seasons.

Discussion

We first evaluated the extent of the carcinogenic liver fluke *O. viverrini* infection areas in which it is endemic by sampling various definitive, reservoir and intermediate hosts. We have also demonstrated an association between the infection of domestic dogs and their owners. A number of epidemiological studies that have included North-East Thailand have revealed high levels of infection for example in Surin; 9.91% (Kaewpitoon et al., 2012c), Nakhonrachasrima; 2.48% (Kaewpitoon et al., 2012b), Mahasarakham; 15.0% (Chaiputcha et al., 2015), Nakhon Panom; 40.9% (Thaewngiew et al., 2014), and it is clear that this is a continuing serious health problem in this region. Some of the effects of liver fluke infection, for example inflammation, periductal fibrosis and proliferative responses, including epithelial hyperplasia, goblet cell metaplasia, and adenomatous hyperplasia (Prakobwong et al., 2009; Prakobwong et al., 2010), may represent pre-disposing lesions that enhance the susceptibility of DNA to carcinogens (Flavell et al., 1980). In our previous report we showed that the intensity and time of infection influenced the severity and pathogenesis of opisthorchiasis in hamsters (Prakobwong et al., 2009; Prakobwong et al., 2010). In the present study, at least 2,265 participants were found to be infected, with the average epg. being 48.9 (range; 12-1,320) with figures of 64.1 epg. (range; 19-1,320) in males and 22.6 epg. (range; 12-474) in females, indicating this is a high risk area for CCA.

This work is also the first to screen a large community population older than 25 years finding that the infection rate was 15.3%. However, because it is well-known from the *O. viverrini* life cycle that it can also be found in domestic reservoir hosts, such as dogs and cats (Aunpromma et al., 2012; Harinasuta and Harinasuta, 1984), the prevalence of *O. viverrini* has also been measured in dogs, cats and cyprinid fish with infection rates of 18.1%, 11.0% and 21.6%, respectively. This study suggests that only treating the liver fluke in humans is unlikely to be successful because the parasite still remains in the natural host, a phenomenon typical of countries in which the parasite is endemic (Aunpromma et al., 2012). The association between *O. viverrini* infection in dogs and their owners suggests that discarded or non-consumptive parts of fish are being passed onto domestic animals. Moreover, even though there was a strong association between *O. viverrini* infection and living near river basins (Figure 1) any prevention and control strategy which only seeks to eliminate it in human subjects will be inadequate. All potential hosts in the community need to be treated.

The established risk factors for liver fluke infection which can lead to enhanced risk of CCA have been identified, including the behavior of consuming raw fish in many traditional fish dishes (Sripa et al., 2007). In addition, the diversity and high populations of cyprinid fish species in North-East Thailand is associated with advantageous environmental conditions (Pinlaor et al., 2013). North-East people enjoy the flavor of uncooked fish and believe that fluke infestations can be eliminated by the use of antihelmintic drugs in the expectation that this will remove the risk of cancer (Wongba et al., 2011), a behavior that leads to continuous reinfection in this region. However, our previous study revealed that reinfection increased the severity of pathogenesis (Pinlaor et al., 2004) and long term liver tissue resorption would only return to normal after praziquantel treatment (Pinlaor et al., 2009). Thus different sociological factors may be at work in each community with different sociologies (Haswell-Elkins et al., 1991). The attitudes of household inhabitants to the selection of food types and their methods of preparation, particularly the choice of fish species to eat and the methods used for their preparation have previously been shown to be important (Chudthaisong et al., 2014). The level of education has been found to be associated with the risk of infection (Chaiputcha et al., 2015) with education to higher than secondary school being a significant protective factor. Most participants in this study had no knowledge of praziquantel but were familiar with albendazole. At least 70% of the infected participants had no history of parasitic treatment and had the behavior to eat raw fish.

The life-cycle of *O. viverrini* depends on intermediate hosts including *Bithynia* sp. (Piratae et al., 2012) and cyprinid fish (Kim et al., 2016) as well as reservoir hosts such as dogs and cats (Aunpromma et al., 2012). We have also determined the prevalence of *O. viverrini* cercaria in *Bithynia* sp., the most common intermediate host. However, the numbers of infected snails detected was less than in other hosts and varied significantly with the season (Namsanor et al., 2015). In cyprinid fish, the

second most common intermediate host of the human liver fluke *O. viverrini* (Pinlaor et al., 2013), the viability of metacercaria in fish dishes has been demonstrated in many species. The average densities measured were in *Henicorhynchus siamensis* (27.7%), *Cyclocheilichthys repasson* (21.9%), *Hampala dispar* (14.1%), and *Barbonymus gonionotus* (6.9%), corresponding to numbers of 17.6, 14.7, 7.9 and less than 2.0 metacercaria per infected fish, respectively. Seasonal populations of *O. viverrini* metacercaria varied between the dry and wet seasons (Sithithaworn et al., 1997). Significantly higher rates of infected fish were found in March and this finding was supported by a previous survey in North-East Thailand (Sithithaworn et al., 1997). The prevalence of metacercariae varied across water resources in North-East Thailand, such as in Nakhon Rachasima and Mahasarakham (Kaewpitoon et al., 2012a; Pinlaor et al., 2013). Also, the metacercarial load in fish was positively associated with infection levels amongst humans in the local population (Sithithaworn et al., 1997). The host-parasite interaction complex may play an important role in determining the pattern of *O. viverrini* distribution. Dogs and cats play key zoonotic roles in and around water resources (Enes et al., 2010) and their infection rates were correlated with the parasite distribution in cyprinid fish (Aunpromma et al., 2012). Our findings show that the infection of humans and their dogs and cats were commonly correlated with living near rivers or lakes. It is possible that the studied areas contain the highest number of people who are eating raw fish in traditional Thai dishes, with some discarded or decayed fish being fed to reservoir hosts.

This study indicates that the epidemiology of *O. viverrini* is maintained in a cycle which is partly hidden in intermediate and reservoir hosts and provides the basic information to allow future effective and sustainable strategies for prevention and control to be developed. In particular this will require health education and behavioral changes amongst affected populations. An earlier report gave information and made recommendations which have been found to be useful to the community (Upatham et al., 1985) and the results of the present study have already been found to be beneficial to the community.

Conflict of interest statement

We declare that we have no conflict of interest.

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