RESEARCH ARTICLE

The Carcinogenic Liver Fluke *Opisthorchis viverrini* among Rural Community People in Northeast Thailand: a Cross-Sectional Descriptive Study using Multistage Sampling Technique

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Abstract

***Opisthorchis viverrini* infection is a serious public health problem in Southeast Asia especially in the northeast and north of Thailand. Therefore, a cross-sectional survey using multistage sampling was conducted from the rural communities of Surin province, Thailand, during September 2013 to July 2014. *O. viverrini* infection was determined using Kato's thick smear technique. Socio-demographic, information resources, and history data were collected using predesigned semi-structured questionnaires. A total of 510 participants completed interviews and had stools collected. Some 32 (6.47%) participants were infected with *O. viverrini*. The rate was slightly higehr 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%). The distribution of high infection was found in Tha Tum (16.7%) and Sankha district (16.7%), followed by Samrong Thap (13.3%), Si Narong (13.33%), and Buachet district (13.33%). 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). Of 72.6% participants who had past histories with stool examination, 17.0% of them had been infected with *O. viverrini* and 43.2% treated with praziquantel. This finding confirmed that *O. viverrini* is still a problem in Surin province, Thailand, and therefore, interventions are urgently required for mass treatment and health education implementation.**

Keywords: Carcinogenic human liver fluke - *Opisthorchis viverrini* - Surin - Thailand

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Introduction

Opisthorchis viverrini, carcinogenic liver fluke, is an endemic in the Lower Mekong Basin, including Thailand, Lao People's Democratic Republic (Lao PDR), 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 (Sripa et al., 2010; Sithithaworn et al., 2012). In Thailand, it is estimated that 6 million people are infected with the *O. viverrini* (Sithithaworn et al., 2012). 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; Sithithaworn et al., 2012). 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 Imsomboon, 2003; Sithithaworn et al., 2012). The *O. viverrini* infection is associated with hepatobiliary diseases including hepatomegaly, cholangitis, cholecystitis, and gallstones (Harinasuta and Vajrasthira 1960; 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) (Thamavit et al., 1978; Harinasuta et al., 1984). The O.viverrini infection in Thailand was the first reported in 1955 (Sadun) and many strategies has been operated over period 1955-

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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). Therefore, to prevent and control of CCA, the screening of *O. viverrini* infection is more required. This cross-sectional survey using multistage sampling was conducted from 17 districts of Surin province, Thailand. This research data could be used to reduce the morbidity and mortality rate of *O. viverrini* infection and CCA, respectively.

Materials and Methods

A cross-sectional survey was conducted in Surin province where is located in the northeast of Thailand, with 450 kilometers from Bangkok (capital) by road and 420 kilometers by train. The province is subdivided into 17 districts. The districts are further subdivided into 158 sub-districts and 2,120 villages, 441,922 houses, and 1,381,761 populations (691,425 males and 690,226 females) (Surin Administrative Organization, Thailand 2013). A survey of *O. viverrini* infection in 17 districts of Surin province was carried out during September 2013 to July 2014.The participants were selected by multistage sampling with cluster size of 30. The estimated sample size required was 510 and was calculated using the following formula:

$$\frac{Z^2(\alpha/2)NP_{(1-p)}\ x\ design\ effect}{Z^2(\alpha/2)P_{(1-p)} + (N-1)d^2}$$

N=population =1,391,635
Z=1.96 95% (α=.05)
Design effect: df=2
D=acceptable margin of error=0.05
P=estimated prevalence rate *O. viverrini* infection=0.161 (Sithithaworn et al., 2012)

Multistage sampling was used to select the samples in this studied, cluster sampling; selected 17 sub-districts from 17 districts, and also the selection of 17 villages registered for each district. Simple random sampling was used to select the samples in the villages with cluster size of 30 (Figure 1). One participant over 4 years of age, who had been living in the sub-district for at least 6 months, was randomly chosen within each household at the time of visiting by a research assistant. Necessary permission from the concerned authorities was taken and a survey was conducted using semi-structured questionnaires included personal data (gender, age, occupation, and education), information resources regarding the *O. viverrini* prevention and control (human and media sources), the histories of stool examination, *O. viverrini* infection, and treatment, Prior informed consent was taken. For those not available in the first interview another visit was made to minimize non-response.

Stools were collected from 30 participants/each districts (who had completed the interviewed) and kept in labeled plastic bags and then transported in an ice box to the laboratory at the Parasitic Disease Research Unit, Department of Pathology, Institute of Medicine, 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 Kato thick smear procedures according to the method of Kato and Miura (1954). 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. Additionally, in order to eliminate fibers or seed, the technique was modified by pressing a 105-mesh stainless steel grid onto the sample which was then filtered, 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

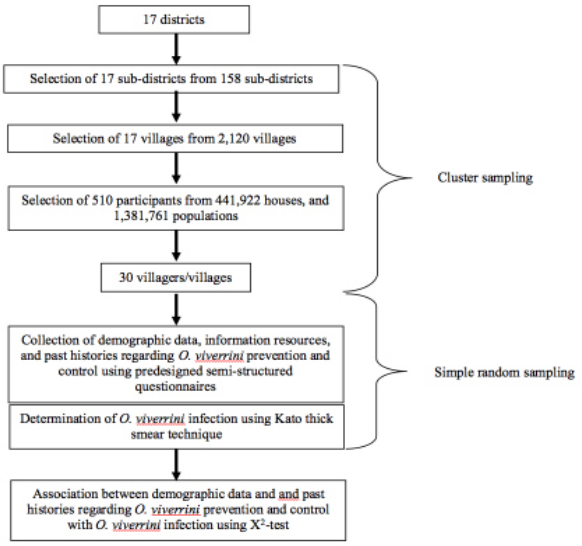

Figure 1. Flowchart of Study Design Regarding a Carcinogenic Liver Fluke *O. viverrini* among Rural Community People in Surin Province, Northeast Thailand

Figure 2. Distribution of *O. viverrini* Infection in Villagers from 17 Districts of Surin Province, Thailand

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 were analyzed with descriptive statistics, and chi-square tests were used to investigate the associations between demographic and histories data with *O. viverrini* infection. Statistical data analysis was carried out using SPSS software version 12.0. The study protocol was approved by Suranaree University Ethical Review Committee, EC55-57.

Results

Of 32 (6.47%) from 510 eligible participants was infected with *O. viverrini*. The *O. viverrini* infection rate was males (6.61%) and females (6.32%). Most of the infected participants were found in the age group 61-70 (19.40%) and 71-80 year old (19.35%), agriculture (10.51%), and primary school level (10.26%), respectively (Table 1). The highest of infection was found in Tha Tum (16.67%), and Sankha district (16.67%), followed by Samrong Thap (13.33%), and Buachet district (13.33%), respectively (Figure 2). Participants were completed the questionnaires about the information resources regarding *O. viverrini* prevention and control. 28.24% of village health volunteer was the main human source, followed by 27.45% of public health officer, and 26.86% of and village headman, were the main information resources regarding *O. viverrini* prevention and control. 41.96% of television was the main media source, followed by 21.37% and 20.59% of village news station and pamphlet, were the main information resources regarding *O. viverrini* prevention and control (Table 2). The past histories with stool examination, *O. viverrini* infection, and praziquantel used, were interviewed. Of 72.55% participants had past histories with stool examination, 17.04% of them had infected with *O. viverrini*, 43.24% participants had treated with praziquantel (Table 3). Chi-square testing indicated that age groups were significantly associated with *O. viverrini* infection (p-value<0.05). The prevalence of *O. viverrini* infection increase with age, and statistically significant with age group 61-70 year old, $X^2(N=67)=10.31$, $p<0.05$, and 71-80 year old, $X^2(N=62)=9.29$, $p<0.05$. The prevalence of *O. viverrini* infection increase with education and occupation, and statistically significant with primary school level, $X^2(N=302)=8.49$, $p<0.05$, and agriculture, $X^2(N=257)=8.13$, $p<0.05$. The proportion of *O. viverrini* infected was slightly higher in males than in females, but the gender different was not statistically significant, $X^2(N=510)=1.10$, $p=0.13$.

Table 1. *O. viverrini* Infection in 17 Villagers of Surin Province, Thailand by Gender, Age, Occupation, and Education

Personal Data	No. of participants	*O. viverrini* infection	%	95% CI
Sex				
male	242	16	6.61	0.10-0.21
female	268	17	6.32	0.11-0.30
Age				
5-10	40	0	0.00	
11-20	50	0	0.00	
21-30	56	1	1.79	
31-40	56	0	0.00	
41-50	58	1	1.72	
51-60	65	3	4.62	
61-70	67	13	19.40*	0.19-0.45
71-80	62	12	19.35*	0.16-0.33
81-90	56	3	5.36	
Occupation				
Agriculture	257	27	10.51*	0.12-0.31
Employee	76	2	2.63	
Student	53	0	0.00	
Unemployable person	43	3	6.98	
Housewife	32	1	3.13	
Trader	28	0	0.00	
Government officer	21	0	0.00	
Education				
Primary school	302	31	10.26*	0.11-0.29
Secondary school	128	1	0.78	
Unlearned	30	1	3.33	
University	21	0	0.00	
Vocational college	29	0	0.00	
Total	510	33	6.47	0.15-0.35

* statistical significant p-value <0.05

Table 2. Information Resources Regarding *O. viverrini* Prevention and Control in Surin Province, Thailand

Information resources	No. of participants	%
Human		
Village health volunteer	144	28.24
Public health officer	140	27.45
Village headman	137	26.86
Nurse	40	7.84
Medical doctor	20	3.92
Teacher	18	3.53
Chief executive of the sub-district administrative organization	11	2.16
Media		
Television	214	41.96
Village news station	109	21.37
Pamphlet	105	20.59
Newspaper	51	10.00
Internet	20	3.92
Poster	11	2.16

Table 3. The Past Histories with Stool Examination, *O. viverrini* Infection, and Praziquantel used, in Surin Province, Thailand

Past histories		No. of participants	%
Stool Examination	Yes	370	72.55
	No	140	27.45
O. viverrini infection	Yes	98	17.04
	No	477	82.96
Praziquantel used	Yes	294	43.24
	No	386	56.76

Discussion

Opisthorchiasis caused by *O. viverrini*, is of considerable public health importance in Southeast Asia,

particularly in Lao PDR and Thailand (Sripa et al., 2010). Human have been infected by ingesting under-cooked fish containing infective metacercariae, this is very common in the northeastern and northern region particularly in rural areas (Sadun 1995; Wykoff et al., 1966; Vichasri et al., 1982; Sithithaworn et al., 1997; Jongsuksantikul and Imsomboon 2003). Although, many program campaign has been operated in Thailand for reduced *O. viverrini* infection over period 1955-2000, the national prevalence of *O. viverrini* infection had fallen but the high prevalence rate is still found in the rural communities of provinces, Northeast (Jongsuksantikul and Imsomboon 2003; Sithithaworn et al., 2012). Recent study, *O. viverrini* infection was 6.76%, this is a first reported a province-wide survey in all age group in rural community of Surin province, Thailand. The highest prevalence was among those between age group 61-70 (19.40%) and 71-80 year old (19.35%); similar to other studied (Kaewpitoon et al., 2012b; Kaewpitoon et al., 2012c; Thaewnongiew et al., 2014). Previous studied in Surin province, 9.91% of elderly were infected with *O. viverrini* (Kaewpitoon et al., 2012c). However, this recent reported shows a higher than the past studied, a large-scale is conducted in this studied due to clear. The proportion of *O. viverrini* infected was slightly higher in males than in females, but the gender different was not statistically significant. People who had an education level with primary school and had an occupation concerning with agriculture, were statistically significantly associated with *O. viverrini* infection, similar to other studied (Thaewnongiew et al., 2014; Suwannahitatom et al., 2013). This figure shows that the middle age and elderly, primary school, and agriculture, are under the need for urgently introduce mass treatment and health education.

The distribution of *O. viverrini* in Surin province was found frequently in 13 from 17 districts, this result indicates that people are still eating fresh fishes containing *O. viverrini* infective stage. A highest prevalence of *O. viverrini* infection was found in Tha Tum, Sankha, Samrong Thap, Buachet and Si Narong district, this result similar to the other studied that had been localization of the risk areas for *O. viverrini* infection. By GIS analysis, Si Narong, Sangkha, Mueang Surin, Non Narai, Samrong Thap, Chumphon Buri, Tha Tum, Buachet, and Rattanaburi to have the highest risk areas in Surin province, Thailand (Rujirakul et al., 2015). A solution for infection prevention needs to be quickly investigated especially for middle-aged and elderly males. When people engage in less risky behaviors, the need and consumption of anti-anthelminthic treatment will be reduced. A health education campaign needs to be organized in areas of the top 5 districts. Present study shows that the village health volunteer, public health officer, and village headman were the important messengers for *O. viverrini* prevention and control. Therefore, the correct of knowledge, attitude, and practices regarding *O. viverrini* information are required. In addition, participants perceived the *O. viverrini* information through television, village news station, and pamphlet, this figure indicates that it may be a useful tool for rural community people improvement via 3 main media. The effectiveness of control programs and its use on a community basis was to prove a major step forward in subsequent control strategies, and region-wide control programs were established from 1987 onwards under the Five-year National Development Plan for 1987-1991. A concise history of opisthiorchiasis control programs in Thailand, and their apparent effectiveness has been detailed by Jongsuksuntigul and Imsomboon (2003) and Sripa et al. (2011). Present study, of 72.55% participants had a history with stool examined, 17.04% had past history with *O. viverrini* infection, and 43.24% had past history with praziquantel used, this result also shown that strategies for the control of *O. viverrini* infection still required for stool examination and treatment of infected cases. Past studied, multiple logistic regression analysis was the used to investigate *O. viverrini* infection factors in the target group, 3,916 Thai residents of northeast Thailand who were 15 or over. The remaining 6 variables were sex, age, water resource habit, having used to medicine for *O. viverrini* infection, having an *O. viverrini* infection records, having salad or fermented fish, were significantly associated to *O. viverrini* infection (Thaewnongiew et al., 2014. The figures show that questionnaire with past histories question may useful for screening of *O. viverrini* in the rural areas.

In conclusion, the present study indicated that *O. viverrini* still found to be a problem in rural community of Surin province, especially in the group of age group over 50 year old, education of primary school, and occupation of agriculture. The infection rate was distributed in 11 districts, therefore, mass treatment and health education are urgently required.

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