RESEARCH ARTICLE

Community-Based Cross-Sectional Study of Carcinogenic Human Liver Fluke in Elderly from Surin Province, Thailand

Soraya J Kaewpitoon[1,2]*, Ratana Rujirakul[1], Naporn Ueng-arporn[1,2], Likit Matrakool[2], Niwatchai Namwichaisirikul[2], Seekaow Churproong[2], Patcharaporn Wongkaewpothong[2], Porntip Nimkuntod[2], Banchob Sripa[3], Natthawut Kaewpitoon[1,2]

Abstract

Background: *Opisthorchis viverrini* infection is a serious public-health problem in Southeast Asia. It is associated with a number of hepatobiliary diseases and the evidence strongly indicates that liver fluke infection is the etiology of cholangiocarcinoma. Objectives: This study aimed to determine *Opisthorchis viverrini* infection in elderly people in Surin province, Northeastern Thailand. Methods: 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. *O. viverrini* infection was determined using Kato's Thick Smear technique and socio-demographic were collected using predesigned semi-structured questionnaires, respectively. Results: A total of 333 elderly including 116 males and 217 females were selected from different study sites. 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%. Chi-square testing indicated that education and occupation were significantly associated with *O. viverrini* infection (P value = 0.02). The distribution of *O. viverrini* infection was found in 11 districts which was covered 64.7% of the studies areas. The highest prevalence was found in Thatum with 39.1%, and followed by Sangkha (24.0%), Buachet (21.1%), Samrong Thap (19.1%), Si Narong (15.0%), and Ratanaburi (13.3%) districts. Conclusion: This findings stress that *O viverrini* is still a problem in Thailand. We confirmed, for the first time, the high endemicity of human *O. viverrini* infections in elderly in Surin province of Thailand, underlying the fact that mass treatment and health education are urgently required.

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

Asian Pacific J Cancer Prev, **13 (9)**, 4285-4288

Introduction

Opisthorchiasis caused by *Opisthorchis 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 undercooked fish containing infective metacercariae, this is very common in the northeastern and northern region particularly in rural areas (Sadun, 1955; Wykoff et al., 1965; Vichasri et al., 1982; Sithithaworn et al., 1997; Jongsuksuntigul and Imsomboon, 2003). The infection is associated with a number of hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis and cholelithiasis (Harinsuta and Vajrasthira, 1960; Harinasuta et al., 1984). The experimental and epidemiological evidences strongly indicate that the liver fluke infection in the etiology of cholangiocarcinoma (CCA); the bile duct cancer (Thamavit et al., 1978; IARC, 1994; Sripa et al., 2007).

In Thailand, it is estimated that 6 million people are infected with the *O. viverrini* (Sithithaworn et al., 2012). The first nationwide survey of the four regions of Thailand during 1980-1981 revealed an overall prevalence of *O. viverrini* infection of 14%; the Northeast (34.6%), the Central (6.3%), the North (5.6%) and the South (0.01%) regions (Jongsuksantikul and Imsomboon, 2003). As a result of intensive and continuous control programs and public health service activities, the average national prevalence of infection has declined to 9.4% in the year 2000 and went down further to 8.7% in the year 2009 (Jongsuksantikul and Imsomboon, 2003). Again, high prevalence of the infection was found in the Northeast (16.6%) followed by the North (10.0%), the Central (1.3%) and the South (0.01%) region of Thailand. Indeed,

*[1]Parasitic Disease Research Unit, Suranaree University of Technology, [2]Institute of Medicine, Suranaree University of Technology, Muang, Nakorn Rachasima, [3]Institute Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand *For correspondence: soraya.k@sut.ac.th*

in the Northeast Region the prevalence in 2009 was similar to that of the previous survey 10 years ago in the year 2000 (15.7%) (Sithithaworn et al., 2012). O.viverrini infection in Thailand, particularly in the North and Northeast Regions, is still prevalent and the highest in the world, however, no detailed data on its prevalence in elderly from Surin province, Thailand have been reported. Therefore, a community-based cross-sectional study was conducted among elderly in Surin province, Thailand. This research data could be used to localize the risk areas to prevent and control the infection.

Materials and Methods

Epidemiological data were obtained from a community-based survey carried out between January and December 2011 in all 17 districts of Surin province, Thailand. A cross-sectional parasitological and questionnaire survey was carried out in Surin province located in northeastern with the total area about 8124 km^2, 450 kilometers away from Bangkok city by cars and 420 kilometers by train. The province is subdivided into 17 districts (amphoe); Mueang Surin, Chumphon Buri, Tha Tum, Chom Phra, Prasat, Kap Choeng, Rattanaburi, Sanom, Sikhoraphum, Sangkha, Lamduan, Samrong Thap, Buachet, Phanom Dong Rak, Si Narong, Khwao Sinarin, and Non Narai, respectively.

The study protocol was approved by Suranaree University Ethical Review Committee. A total of 333 elderly (60 year old and over), 116 male and 217 female were randomly selected. Necessary permission from the concerned authorities was taken and a survey was conducted using pretested semi-structured questionnaires. 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 individualized elderly (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.

A total of 333 stool specimens were collected and examined the *O. viverrini* by the modified Kato thick smear procedures. The modified Kato thick smear was prepared and processed according to the method of Kato and Miura (1954). 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 objects were subsequently examined under a high-power (40x) objective. The stool samples were preserved in 10% formalin for later confirmation, if needed. Every positive case of *O. viverrini* infection identified by the modified Kato method was confirmed by 2 exerted parasitologists before a definitive diagnosis was established. *O. viverrini* egg was shown in Figure 1. Patients who infected with other known parasitic were treated with anti-parasitic drugs and also attended the health education.

Statistical data analysis was carried out using SPSS software version 12.0. Chi square test was performed to determine association between socio-demographic and *O. viverrini* infection.

Results

Results are shown in Tables 1 to 3. The total of elderly was 333 from 17 districts of Surin province, Thailand. There were 65% females and 34.83% males and the majority of age in the range of 60-70 year old, 48.65%. Most of them had the education in the primary level (86.47%), and they were a farmer (58.26%). All of general characteristics of elderly who were selected to this studied showed in Table 1. Overall intestinal parasitic infection was 16.23%, predominantly in *O. viverrini* (9.91%) and followed *S. stercolaris* (4.80%) and hookworm (1.50%), respectively (Table 2). Mixed infection was 1.20%, classified to *O. viverrini*+*S. stercolaris* (0.6%) and *O. viverrini*+hookworm (0.6%), respectively. The *O. viverrini* infection was higher in male (13.79%) more than female (7.83%), and frequently in the age group 60-70 year old with 14.20%. Chi-square testing was analyzed on the

Figure 1. The *O. viverrini* egg was determined from the Elderly Selected from Various 17 Districts, Surin Province, Thailand (Magnitude 1000x).

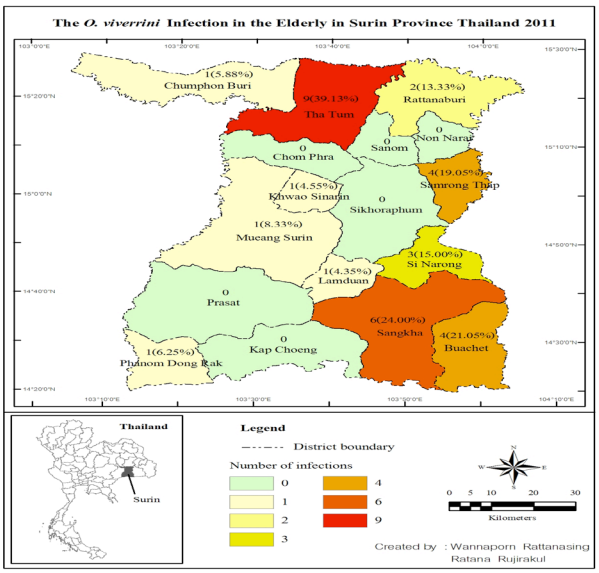

Figure 2. The Prevalence of *O. viverrini* in the Elderly Selected from Various 17 Districts, Surin Province, Thailand.

Table 1. Characteristics of the Elderly from Various 17 Districts, Surin Province Thailand

Variable		%(No. of infected/examined)	
Sex	Male	13.79	(16/116)
	Female	7.83	(17/217)
Age	60-70	14.20	(23/162)
	70-80	9.64	(8/83)
	80-90	25.00	(2/8)
	>90	0	(0/0)
	unknown	0	(0/80)
Education level	None	7.50	(3/40)
	Primary	10.42	(30/288)*
	Secondary	0	(0/4)
	College	0	(0/1)
Occupation	Farmer	15.46	(30/194)*
	Government officer	0	(0/1)
	Employed	0	(0/4)
	Unemployed	2.00	(2/100)
	House work	2.94	(1/34)

*p value=0.02

Table 2. Intestinal Parasitic Infection in the Elderly Selected from Various 17 Districts, Surin Province Thailand

Parasitic species	% (No. of patient infected/examined)	
Opisthorchis viverrini	9.91	(33/333)
Strongyloides stercolaris	4.80	(16/333)
Hookworm	1.50	(5/333)
Total	16.23	(54/333)

Table 3. The Prevalence of *O. viverrini* Found in the Elderly Selected from Various 17 Districts, Surin Province, Thailand

District sites	% (No. of patient infected/examined)	
Tha Tum	39.13	(9/23)
Sangkha	24.00	(6/25)
Buachet	21.05	(4/19)
Samrong Thap	19.05	(4/21)
Si Narong	15.00	(3/20)
Rattanaburi	13.33	(2/15)
Mueang Surin	8.33	(1/12)
Phanom Dong Rak	6.25	(1/16)
Chumphon Buri	5.88	(1.17)
Khwao Sinarin	4.55	(1/22)
Lamduan	4.35	(1/23)
Prasat	0	(0/23)
Sanom	0	(0/22)
Kap Choeng	0	(0/21)
Non Narai	0	(0/19)
Sikhoraphum	0	(0/18)
Chom Phra	0	(0/17)
Total	9.91	(33/333)

education, a significant associated between primary level and *O. viverrini* infection (P value = 0.02). In addition, the occupation was calculated that a significant associated was seen between the farmer and *O. viverrini* infection (P value = 0.02).

The infection rates of *O. viverrini* in 17 districts for the year-round survey are shown in Table 3 and Figure 2. The distribution of *O. viverrini* in Surin area was 64.71%, covered 11 districts. The highest infection rate was found in Tha Tum district with 39.13%, and followed Sangkha (24.00%), Buachet (21.05%), Samrong Thap (19.05%), Si Narong (15.00%), and Ratanaburi (13.33%) districts, while in 6 districts emphasized Prasat, Sanom, Kap Choeng, Non Narai, Sikhoraphum, and Chom Phra were not fond the *O. viverrini* infection in the elderly subjects.

Discussion

The Helminthiasis control program started in 1950 included opisthorchiasis control in some high risk areas. The main liver fluke control strategies comprise of three interrelated approaches, namely stool examinations 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 the improvement of hygienic defecation for the interruption of disease transmission (Jongsuksuntigul & Imsomboon, 2003). The first nationwide survey of the four regions of Thailand during 1980–1981 revealed an overall prevalence of *O. viverrini* infection of 14%; the Northeast (34.6%), the Central (6.3%), the North (5.6%) and the South (0.01%) regions. As a result of intensive and continuous control programs and public health service activities, the average national prevalence of infection has declined to 9.4% in the year 2000 and went down further to 8.7% in the year 2009 (Jongsuksantikul and Imsomboon, 2003; Sithithaworn et al., 2012). Based on the data in 2009, *O. viverrini* infection in Thailand, particularly in the North and Northeast Regions, is still prevalent and the total number of opisthorchiasis cases is estimated to be more than 6 million, the highest in the world (Sithithaworn et al., 2012).

Although, the helminthiasis control program have been started for a long time but it still prevalent and found the *O. viverrini* in human and rural community. The present study confirmed, for the first time, the high endemicity of human *O. viverrini* infections in elderly in Surin provice of Thailand where locate in the northeastern region. The prevalence was assessed as high as almost 40% among the surveyed elderly in some districts such as That um, Sangkha, and Buachet, respectively. Overall prevalence of *O. viverrini* was 9.91%, this prevalence is similar to that of the previous survey in other provinces of the northeastern region A high prevalence of the infection was found in the Northeast (16.6%) followed by the North (10.0%), the Central (1.3%) and the South (0.01%) region of Thailand (Sithithaworn et al., 2012). The *O. viverrini* infection was found higher in male (13.79%) more than female (7.83%), this figure shows that male may caused by behavior of consumption and social participation. The *O. viverrini* infection was found predominantly in elderly especially age ranked 60-70 year old. This age-prevalence pattern similar with other studies has been observed for *O. viverrini* in Thailand (Upatham et al., 1982; Sithithaworn et al., 2003; Sriamporn et al., 2004; Kaewpitoon et al., 2008). The prevalence of infection rises rapidly with age up to adult hood and remains relatively high, thereafter, the relationship of prevalence to age being a function of "Koi pla" consumption. The intensity of infection (faecal egg output) in both males and females rises steadily in

early life, reaches highest in the 55-64 years age group, the reported cases were found common in 35-44, 45-54, 55-64 and 65+, 1.48, 2.06, 2.21 and 1.43 per population, respectively.

Our study indicates the education and occupation were significant associated between primary level and farmer with *O. viverrini* infection. This result was similar to that previous studied about the knowledge related to liver fluke infection in Thailand (Kaewpitoon et al., 2007). They may have a low knowledge and lack of data information on the *O. viverrini* transmission, prevention and control. In further studies, we should be following the patients who infected with *O. viverrini* about their knowledge, attitude, and practice. The distribution of *O. viverrini* in Surin area was 64.71%. The highest infection rate was found in Tha Tum district with 39.13%, and followed Sangkha, Buachet Samrong Thap, Si Narong, and Ratanaburi districts. This dramatically data indicates an epidemic areas for Surin province and need urgently to mass treatment and diagnosis of Cholangiocarcinoma.

By the way, we have examined for intestinal parasitic infections and found that *S. stercolaris* (4.80%) and hookworm (1.50%) infected in elderly. Mixed infection was also found in the subjects, *O. viverrini*+S. stercolaris, and *O. viverrini*+hookworm, respectively. Hookworm and *S. stercolaris* larvae can enter through the skin of host, typically on the foot, and travel up through the bloodstream into the lungs. Hookworm and *S. stercolaris* disease can produce an itchy rash, coughing with or without bloody sputum. When present in the intestines, hookworm and *S. stercolaris* infection usually does not produce any recognizable symptoms. However, some people may experience diarrhea, abdominal pain, intestinal cramps, and nausea. Chronic or persistent hookworm and *S. stercolaris* disease can cause anemia due to blood loss, especially in people with poor health or in pregnant women (Ball and Michael, 1991), therefore, should be concerned to treat and prevent in elderly.

In conclusion, our study showed the update prevalence of *O. viverrini* in elderly in Surin Province, Thailand. This findings stress that O viverrini are still a problem in Thailand, we present study confirmed, for the first time, the high endemicity of human *O. viverrini* infections in elderly in Surin provice of Thailand, therefore mass treatment and health educations are urgently required.

Acknowledgements

We would like to thank the National Research Council of Thailand for supported the grants Fiscal year 2011.

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