RESEARCH ARTICLE

Prevalence of OV Infection in Yasothon Province, Northeast Thailand

Phubet Saengsawang[1,2], Supannee Promthet[1]*, Peter Bradshaw[1]

Abstract

A liver fluke, *Opisthorchis viverrini* (OV), is the major cause of the high incidence of cholangiocarcinoma in Thailand. The prevalence of OV 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 uncooked 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. This paper is the first report of prospective research project designed to monitor the impact of the national control program in rural communities located in a northeastern province and at high risk of OV infection. The participants in this initial survey were 1,569 villagers, aged 20-65 years, living in two subdistricts of Yasothon Province. Stool examinations showed that 38.68% were infected with OV. Males were slightly more likely to be infected than females, but the difference was not statistically significant. Infection was found to be positively associated with age in both males and females. The preliminary data indicate that the population selected for study is suitable for the purpose of the monitoring project.

Keywords: Prevalence - *Opisthorchis viverrini* - control programs - rural Thailand

Asian Pacific J Cancer Prev, **13**, 3399-3402

Introduction

The liver fluke, *Opisthorchis viverrini* (OV), continues to be the source of a serious public health problem in Thailand. Over six million Thai people are estimated to be infected, and the highest prevalence rates are found in the north and northeastern regions (Sripa et al., 2011). Whereas OV infection is usually asymptomatic, the organism has been classified as a type-1 carcinogen for many years (IARC, 1994) and is a major risk factor for the subsequent development of cholangiocarcinoma (Watana and Watana, 2002; Poomphakwaen et al., 2009; Songserm et al., 2012), which is a usually fatal form of liver cancer (Khan et al., 2005). The incidence rate of this disease in Khon Kaen, a province in Northeast Thailand with historically very high rates of OV infection, has been higher than in any other part of the world (Sripa et al., 2007).

The life cycle of OV is well-known, but briefly the definitive hosts are man and other animals, such as dogs, rats and especially cats (Enes et al., 2010; Aunpromma et al., 2012) who eat raw, uncooked or under-fermented freshwater cyprinoid fish. Eggs of the liver fluke are shed in faeces and are ingested by the *Bythinia* snail. The eggs encyst, reproduce asexually and are then released into water as free-swimming cercariae. These penetrate the skin of mainly cyprinoid fish and encyst in the fins, skin and muscle to become metacercariae. When the raw, uncooked or under-fermented infected fish are eaten by definitive hosts, the metacerariae excyst and develop as adult liver flukes in the bile duct.

The geographic pattern of OV infection rates in Thailand is very uneven (Shin et al, 2010), but high rates are more likely in rural than urban environments, especially in wetlands and agricultural areas (Wattanayingcharoenchai et al., 2011) where there are rivers, lakes, man-made watercourses for irrigation, ponds for aquaculture and rice-fields and where the food-culture of the local people includes the consumption of raw or undercooked fish (Grundy-Warr et al., 2012). Within Northeast Thailand, a region where the parasite has remained highly endemic, OV prevalence in 2009 varied considerably from one province to another with infection rates ranging from 4.6% to 60.8% (Sithithaworn et al., 2012). High variability has also been reported between the different districts within a province; for example, the age and gender adjusted proportion of the population infected in the 20 district of Khon Kaen Province varied between 10.0% and 70.9% (Sriamporn et al 2004). However, the crude data were collected over the period 1990 to 2001, and it was not known how far the differences might have also reflected changes in prevalence rates over that time.

[1]*Department of Epidemiology, Faculty of Public Health, Khon Kaen University,* [2]*Yasothon Health Office, Yasothon Province, Thailand*
**For correspondence: supannee@kku.ac.th*

Strategies for the control of OV infection have involved identification by stool examination and treatment of infected cases with praziquantel, health education campaigns to encourage the consumption of only properly cooked fish, and the improvement of sanitation and discouragement of defaecation in wetland areas to prevent transmission by human hosts (Jongsuksuntigul and Imsomboon, 1998; Wattanayingcharoenchai et al., 2011). A concise history of opisthiorchiasis control programs in Thailand and their apparent effectiveness is provided by Jongsuksuntigul and Imsomboon (2003) and Sripa et al. (2011). To summarise, the first control programs in Thailand were operated prior to the development of praziquantel in the period 1950-1958, but control units were established in only five provinces and were dissolved when US aid ceased in 1958. However, the elements of the control program persisted and were part of rural health development. The demonstrated effectiveness of praziquantel in 1980 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. By 1992 a national control program was operating in 42 provinces. The control program is now integrated with the rural health services, which cover all provinces, and the Ministry of Public Health has set a target prevalence of 5% by 2016. Over the period 1981-2001 a variety of different surveys indicated that the national prevalence of OV infection had fallen from 63.6% to 9.6%, but the most recent a data indicate the prevalence rate in the Northeast region remains high at 16.6% and had not decreased over the previous decade (Sithithaworn et al., 2012) .

An accurate evaluation of the impact of the national control program is difficult on the basis of cross-sectional surveys performed on diverse populations in disparate geographical locations using different sampling techniques and methods of OV stool examination. Furthermore, such surveys carried over time do not take into account possible confounding factors, which may affect the prevalence rates, such as changes in the pattern of agricultural use of land in wetland areas, changes in land use due to industrial and residential development, and changes in the number of agricultural workers required because of increasing mechanization of farming practices.

The aim of the present study was to select an area of Northeast Thailand where at least moderate levels of current OV infection were expected and where changes due to the national program could be assessed, controlling for variables which were likely to affect infection rates, but could not be attributed to factors unrelated to the substance of the national control program. This study provides a baseline prevalence of OV for future prospective research into the efficacy of the national control program. No additional control measures will be used.

Materials and Methods

This cross–sectional survey was conducted in Yasothon Province during January – March 2012. Yasothon Province covers an area of 4,161 km2 in Northeast Thailand and had a population of 539,257 in 2010. While in the northern part of the province the terrain is mainly composed of plains with low hills, the southern area is the lowland of the Chi River and contains swamps and many ponds. The most recent available data indicate that the OV infection rate for the province is 22.5% compared with a rate of 15.7% for the entire Northeast region (Sithithaworn et al., 2012) and that the mortality rate for cholangiocarcinoma is 39.9/100,000 compared with 43.6/100,000 (Sripa and Pairojkul, 2008).

The study areas were two subdistricts, which were selected by multistage sampling from the nine provincial districts. Both the subdistricts were rural areas in the southern half of Yasothon. The first study area was Kut Kung in the district of Kham Khuean Kaeo. This subdistrict is about 28 kms from provincial capital, covers an area of 35.0 kms2 and is composed of seven villages and 913 households. The population between 20 and 65 years of age is 3,026, and 85% are involved in agriculture. Water from the Chi River and four reservoirs is used for irrigation. The second study area was Hua Mueang in the district of Maha Chana Chai. This subdistrict is about 46 kms from the provincial capital, covers 36.5 kms2, and is composed of 14 villages and 1,635 households. The population between 20 and 65 years of age is 4,907, and 80% are involved in agriculture. Irrigation is provided by water from the Chi River, two reservoirs and canals around the villages.

All people, who had resided in either study area for at least six months and were between 20 and 65 years of age, were invited to participate and agree to provide samples for a stool examination. Stools were examined within two days of collection using the Kato thick smear technique (WHO, 1991). Villagers (n=15), who reported taking praziquantel in the week prior to stool collection, were excluded.

Data analyses involved the use of basic descriptive statistics and chi-square tests for the age/gender comparisons. Statistical significance was set at $p<0.05$.

The research was approved by the Khon Kaen University Ethics Committee for Human Research (reference no. HE542213)

Results

The survey results are summarised in Table 1. A total of 1,569 villagers (739 males and 830 females) participated in the survey. The sample represented 19.78% of the total population of the two villages in the 20-65 year age group. The stool examination results showed that 38.68% of the participants were infected with OV. The proportion of infected males (40.05%) was slightly higher than the infection rate in females (37.47%), but the gender difference was not statistically significant, X^2 (1, N=1,569) = 1.10, p = 0.294 (Table 1). The prevalence of OV infection increased with age, and statistically significant associations with age were found in both males, X^2 (2, N=739) = 25.11, p<0.0001) and females, X^2 (2, N=830) = 10.52, p = 0.005.

Table 1. OV Infection in Villagers by Gender and Age

Variables	No. of villagers examined	No. of OV positives	%OV positives	95%CI
Age-group in years (Both sexes)				
20-35	248	62	25.00	0.19-0.31
36-55	850	323	38.00	0.35-0.41
56-65	471	222	47.13	0.43-0.52
Total	1,569	607	38.68	0.36-0.41
Age-group in years (Males)				
20-35	124	29	23.39	0.16-0.32
36-55	388	152	39.18	0.34-0.44
56-65	227	115	50.66	0.43-0.57
Total	739	296	40.05	0.36-0.44
Age-group in years (Females)				
20-35	124	33	26.61	0.19-0.35
36-55	462	171	37.01	0.33-0.42
56-65	244	107	43.85	0.37-0.50
Total	830	311	37.47	0.34-0.41

Discussion

This study represents a prospective 'work in progress' and is intended to provide baseline information for future follow-up surveys to monitor infection (and especially re-infection) rates. The report on the next survey will include detailed socio-demographic information about the participants, and an additional study with a subsample of the villagers is underway to determine prevailing knowledge, beliefs and behaviours relevant to OV infection.

The current prevalence rates in rural Yasothon are high and offer room for substantial improvements. The rates also indicate that the results of the national control program in rural parts of Northeast Thailand are disappointing. For reasons as yet not completely understood, the program does not appear to be having much impact in these areas. Similar indications of deficiencies in the national control program have been seen in rural communities elsewhere. For example, Rangsin et al. (2009) completed a two-year prospective survey of villagers of Chachoengsao Province in the Central region east of Bangkok and reported that the prevalence rate of OV infection increased from 21.3% to 26.2%. The second survey result was based only on those negative for OV in the first survey. Certainly, it is hard to avoid the conclusion that existing control programs are not working in rural areas due to underfunding and limited outreach, especially in the neglected Northeast and where people continue to eat raw, semi-cooked or fermented fish dishes such as *koi pla, lap pla,* and *pla som* (Grundy-Warr et al., 2012).

The results of the present study suggest a slight, but non-significant higher prevalence of OV infection in male rural-dwellers than in females. There was also a strong positive correlation with age in both males and females, especially in males. Analyses of data from the Khon Kaen cohort study have shown a higher prevalence of OV infection in males than females and increasing prevalences with age in females, but not in males (Sriamporn et al.,2004; Sriamporn et al., 2005). In the present study, prevalence was correlated with age in both sexes and the association appeared stronger in males than females. Studies of village communities elsewhere in Thailand have reported results consistent with the Yasothon data. Rhongbutsri and Kitvatanachai (2002) found prevalence was highest in villagers over 50 years old (no breakdown by sex reported), and the results of Rangsin et al. (2009) also showed a non-significant higher prevalence in males and increasing rates of infection with age. While the higher rates of infection in the older age groups puts these people at greater risk of cholangiocarcinoma and can result in serious losses of income for families when they develop the disease during their working lives, one positive aspect is the suggestion that dietary habits are changing and that villagers in the younger age groups are less likely to subscribe to the traditional rural practice of eating raw or undercooked fish products.

A major limitation of community monitoring studies is that the process of data collection and the very presence of a research team can prime changes in the study population and local public health service delivery, which would not otherwise have occurred and therefore reduce the generalisability of the findings. Attempts will be made to reduce threats to external validity and to document where these are likely to have occurred.

The sample of villagers participating in this study is considered suitable for the purpose of the monitoring project. The participants represent approximately 20% of the target village populations, there is a good distribution across the different gender and age groups, and the current OV infection rate and its correlation with age are consistent with the findings of various cross-sectional surveys in the region. While the project design is less than optimal, liver-fluke induced cholangiocarcinoma has devastating personal and socio-economic consequences, and those exposed to the highest risk are the low-income, less privileged members of the community. Attempts to track the infection rates and effectiveness of the national control program in the areas of most risk is major priority if attention is to be drawn for the need of additional resources to improve prevention strategies in these areas.

Acknowledgements

This study was supported by the National Research University Project of Thailand, through the Center of Excellence in Specific Health Problems in the Greater Mekong Sub-region (SHeP-GMS), Khon Kaen University (research grant no. NRU542005), KKU Graduate School, and Research and Training Center for Enhancing Quality of Life of Working Age People.

References

Aunpromma S, Tangkawattana P, Papirom P, et al (2012). High prevalence of *Opisthorchis viverrini* infection in reservoir hosts in four districts of Khon Kaen Province, an opisthorchiasis endemic area of Thailand. Parasitology International, **61**, 60-4.

Enes JE, Wages AJ, Malone JB, Tesana S (2010). Prevalence of *Opisthorchis viverrini* infection in the canine and feline hosts in three villages, Khon Kaen Province, northeastern Thailand. *Southeast Asian J Trop Med Publ Health*, **41**, 36-42.

Grundy-Warr C, Andrews RH, Sithithaworn P, et al (2012). Raw attitudes, wetland cultures, life-styles: Socio-cultural dynamics relating to *Opisthorchis viverrini* in the Mekong basin. *Parasitology Int*, **61**, 65-70.

IARC (1994). Schistosomes, liver flukes and Helicobacterpylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum*, **61**, 1-241.

Jongsuksuntigul P, Imsomboon T (1998). Epidemiology of opisthorchiasis and national control program in Thailand. *Southeast Asian J Trop Med Public Health*, **29**, 327-32.

Jongsuksuntigul P, Imsomboon T (2003). Opisthorchiasis control in Thailand. *Acta Tropica*, **88**, 229-32.

Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD (2005). Cholangiocarcinoma. *Lancet*, **366**, 1303-14.

Poomphakwaen K, Promthet S, Kamsa-ard S, et al (2009). Risk factors for cholangiocarcinoma in Khon Kaen, Thailand: a nested case-control study. *Asian Pac J Cancer Prev*, **10**, 251-7.

Rhongbutsri P, Kitvatanachai S (2002). Survey of the fluke infection rate in Ban Kok Yai village, Khon Kaen, Thailand. *J Trop Med Parasitol*, **25**, 76-8.

Shin H, Oh J, Masuyer E, Curado M, et al (2010). Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Sci*, **101**, 579-85

Sithithaworn P, Andrews RH, Van De N, et al (2012). The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitology Int*, **61**, 10-6.

Songserm N, Promthet S, Sithithaworn P, et al (2012) Risk factors for cholangiocarcinoma in high-risk area of Thailand: role of lifestyle, diet and methylenetetrahydrofolate reductase polymmorhisms. *Cancer Epidemiology*, **36**, 89-94.

Sriamporn S, Pisani P, Pipitgool V, et al (2004). Prevalence of *Opisthorchis viverrini* infection and incidence of cholangiocarcinoma in Khon Kaen, Northeast Thailand. *Trop Med Int Health*, **9**, 588-94.

Sripa B, Bethony JM, Sithithaworn P, et al (2011). Opisthorchiasis and Opisthorchis-associated cholangiocarcinoma in Thailand and Laos. *Acta Trop*, **120**, 158-68.

Sripa B, Kaewkes S, Sithithaworn P, et al (2007). Liver fluke induces cholangiocarcinoma. *PLoS Med*, **4**, 201.

Sripa B, Pairojkul C (2008). Cholangiocarcinoma: lessons from Thailand. *Curr Opin Gastroenterol*, **24**, 349-56.

Watanapa P, Watanapa WB (2002). Liver fluke-associated cholangiocarcinoma. *Br J Surg*, **89**, 962-70.

Wattanayingcharoenchai S, Nithikathkul C, Wonsaroj T, Royal L, Reungsang P (2011). Geographic information system of *Opisthorchis viverrini* in northeast Thailand. *Asian Biomedicine*, **5**, 687-91

World Health Organization (1991). Basic laboratory methods in medical parasitology. World Health Organization, Geneva pp 25-8.