RESEARCH ARTICLE

Re-examination of *Opisthorchis viverrini* Infection in Northeast Thailand

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Abstract

Background: Liver fluke infection caused by the parasite *Opisthorchis viverrini* (*O. viverrini*), a human carcinogen, is endemic in north-eastern Thailand and remains a major health problem. Objectives: The objectives of the study were to (1) resurvey the prevalence of *O. viverrini* infection in a field site from the Khon Kaen Cohort Study (in newly recruited subjects as well as previous cohort subjects surveyed in 1992); (2) investigate how subjects' lifestyle habits and their exposure to health promotion initiatives influence changes in prevalence of *O. viverrini* infection. Materials and Methods: The prevalence of *O. viverrini* infection in the cohort subjects (as well as new subjects) was investigated using faecal egg counts. Information on demographic factors, lifestyle and awareness of health promotion initiatives were obtained through questionnaires. Results: *O. viverrini* infection rates in the same individuals of the cohort were lower in 2006 than in 1992. Also, by studying the period effect, the current 35-44 year olds had a 12.4% (95% CI 3.9% to 20.9%) lower prevalence of *O. viverrini* infection than the 35-44 year olds in 1992 (24.2% versus 11.8%). Lifestyle choices showed that smoking and alcohol consumption were associated with an increased chance of acquiring *O. viverrini* infection with adjusted odds ratios of 10.1 (95%CI 2.4-41.6) and 5.3 (95%CI 1.2-23.0), respectively. Conclusions: Our study has demonstrated that although the prevalence of *O. viverrini* infection over a 14-year period has decreased, unhealthy lifestyle was common with smoking and alcohol consumption being associated with increased chances of infection, emphasising the double burden of disease which developing countries are facing.

Keywords: Opisthorchis viverrini infection - Cholangiocarcinoma - *O. viverrini* antibody - lifestyle

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Introduction

Opisthorchis viverrini, a trematode parasite which has been classified as a human carcinogen for cholangiocarcinoma (CCA) (Bouvard et al., 2009; IARC, 2012), remains a major global health problem (Andrews et al., 2008; Fürst et al., 2012; Murray et al., 2012). Consumption of raw or undercooked cyprinoid fish, an intermediate host for *O. viverrini*, is the main cause of infection (Sadun 1955, Wykoff 1965, WHO 1995). Through chronic infection, carcinogenesis is thought to be multifactorial involving the parasite as well as host factors (Honjo et al., 2005; Sripa et al., 2008; Songserm et al., 2012).

Infection with *O. viverrini* is endemic in northeast Thailand, where aquaculture is widespread and consumption of raw fermented fish with rice forms a staple diet (Upatham 2003). In the 1990s, the overall prevalence of *O. viverrini* infection in the north-eastern province of Khon Kaen was 24.5%, ranging from 2.1% to 70.8% in its different districts (Sriamporn et al., 2004). This high prevalence accounts for the extraordinarily high incidence of CCA with age-standardised rates of 93.8 to 317.6 per 100,000 person-years in 1988-1989 (Vatanasapt et al., 1992). In contrast, the age-standardised rates of CCA generally range from 1-2 per 100,000 within Asian populations in non-endemic parts of the world (Parkin et al., 1993).

Region-wide liver fluke control programmes were initiated in Thailand in 1987 as part of a governmental public health initiative. Health promotion approaches vary across provinces; strategies include the provision of the anti-helminthic praziquantel for affected cases, promotion of health education and development of environmental sanitation (Jongsuksuntigul et al., 2003; Sithithaworn, 2003; Sripa et al., 2015). The Ministry of Public Health in Thailand aims to reduce the prevalence of *O. viverrini* infection to less than 5% by 2016.

Although praziquantel is effective, re-infection rates are very high; rates of nearly 90.0% within a year have been recorded in Khon Kaen (Upatham et al., 1988). Ingrained socio-cultural habits, accessibility of praziquantel and

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unsustainable control programmes are thought to have contributed to higher infection rates (Kaewpitoon et al., 2007; Grundy-Warr et al., 2012; Saengsawang et al., 2013; Sithithaworn et al., 2012; Thaewnongiew et al., 2014). This has translated to the continued persistence of CCA in the region (Parkin et al., 2002; Attasara et al., 2012).

To date, there have not been any longitudinal studies investigating factors associated with the acquisition and persistence of *O. viverrini* infection in individuals, which may provide useful clues for preventive action. In the present study we investigate the *O. viverrini* infection status in a group of individuals who had been recruited into a cohort study, (Sriamporn et al., 2005) some 14 years earlier, and examine lifestyle, demographic information and awareness of health promotion initiatives for associations with current as well as the change of *O. viverrini* infection rates.

Materials and Methods

Study population

The study subjects were 469 residents from one sub-district (Wang Sang sub-district) of Chonnabot district within the wider Khon Kaen province. 272 had been recruited into the Khon Kaen cohort study in 1992: 149 at ages 35-44 years (Group 1) and 123 at ages 45-54 years (Group 2). The latter two groups were aged 49-68 years at the time of the present study in 2006. The remaining 197 subjects, aged 35-44 (Group N), were newly recruited (94.0% of those invited); these subjects were too young to have been enrolled into the cohort study in 1992. Figure 1 illustrates the three groups in the study.

Examination and data collection

Eligible subjects were invited to attend a health survey, the purpose of which was explained to them prior to, as well as during, the visits. All subjects willing to participate provided informed consent. Information on demographic factors, lifestyle (tobacco and alcohol use, past infection with *O. viverrini* and treatment with anti-helminthic), and awareness of health promotion initiatives for *O. viverrini* prevention were obtained by questionnaire. Physical examination (height, weight and blood pressure) and biological sampling (blood and stools) were also performed. During the visits, participating subjects received a health promotion presentation after completing the survey. Blood and faecal specimens were stored on ice and were delivered on the same day to the laboratory. The questionnaire was the same as that used in the initial recruitment into the cohort in 1992 (Sriamporn et al, 2005), but excluded the extensive dietary history, and added questions on the awareness of health promotion initiatives. Incomplete and incorrectly completed questionnaires were verified and modified with subjects and staff at the village health centres.

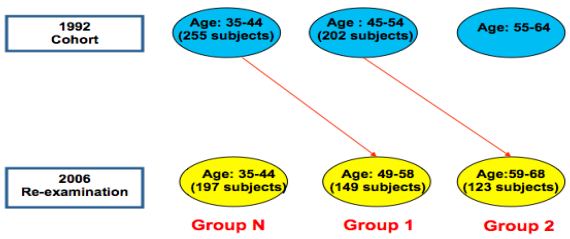

Figure 1. Diagram Showing the Study Populations. Groups coloured in yellow indicate the subject groups recruited for the present resurvey and the numbers in red indicate their respective groupings for analysis

For subjects in Groups 1 and 2, the results obtained in 1992 were compared with those obtained in 2006

Laboratory diagnosis of O. viverrini

The methods for diagnosis of *O. viverrini* infection were the same in 1992 and 2006. Intensity of infection was quantified by faecal egg count, using the modified quantitative formalin/ethyl acetate concentration technique. Anti-*O. viverrini* IgG antibody titres in blood (serum) were measured using optical density (OD) values based on the indirect ELSIA method (Elkins et al., 1991). For the indirect ELISA method, *O. viverrini* antigen (5 μg of crude antigen/ml (100 μl/well)) prepared from worms was used to coat microtitre plates (MaxiSorp; Nunc, Roskilde, Denmark). Human serum samples (diluted to 1:200) were then added followed by peroxidase-conjugated goat antihuman IgG (Zymed, San Francisco, USA) (diluted to 1:10000).

For *O. viverrini* IgG titres, 269 subjects (in groups 1 and 2) had serum specimens from both 1992 and 2006. Of those taken in 1992, 162 had been analysed at the time. The remainder (107), together with the 269 repeat specimens taken in 2006, were analysed in 2007. Because the O.viverrini antigen used for analysis was different on the two occasions, there was a significant difference between the OD values of the corresponding two batches of 1992 specimens. In order to study change in antibody titres, the values for specimens measured previously in 1992 were corrected by log transformation and the adjusted values used for comparative analysis.

Adjustement of O. viverrini antibody titres for comparative analysis

In order to study the change in titres in the whole population, the estimated values for 1992 measured in that same year must be corrected to a value that might have been expected, had they been analysed in 2007.

The values were converted to LOG titres, which makes the distribution of their values (in the old and new analyses) approximately normal. Then, to the (log) values for the old (1992) analyses, we add the difference of the log means of 2006 and 1992 (from the 2007 analyses).

$$LOG\ (test_{07})1992 = LOG\ (t_o)1992 + [LOG\ (X_{07})1992 - LOG\ (X_{07})2006]$$

Where

$(test_{07})1992$ is the estimated (2007) value for 1992, (for those specimens actually in the old batch)

$(t_o)1992$ is the titre in the original batch for 1992

$(X_{07})1992$ is the mean titre of the 2007 specimens for 1992

$(X_{07})2006$ is the mean titre of the 2007 specimens for 2006

The distribution of values for LOG $(test_{07})1992$ was then adjusted so that the mean remains constant, but the distribution has the same standard deviation as that of the 1992 specimens, measured in 2007

Data analysis

Descriptive statistics on demographic and lifestyle information were reported. The Chi squared (X^2) test of proportions was used to assess differences measured between different groups and the McNemar's test was used to assess differences between the same cohort subjects between 1992 and 2006. Analysis was based on the 0.05 level of statistical significance with 95% confidence intervals (CIs) of proportions reported. Multivariate analysis using logistic regression models adjusted for variables and their association with current and persistent *O. viverrini* infection. Analysis was done using STATA (version 10, StataCorp, 2007).

Ethical approval

Ethical approval to conduct the study was obtained from the Khon Kaen University Ethics Committee for Human Research in accordance with the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines (reference no: HE490343).

Results

In 2006, the overall prevalence of *O. viverrini* infection at ages 35-68 years was 9.3%. Table 1 shows the number of subjects positive and negative for *O. viverrini* in 1992 and 2006 in the two groups of subjects studied in both years. In group 1, the prevalence was 6.5% in 2006 (compared with 23.7% in 1992); in group 2 it was 7.6% in 2006 (compared with 23.7% in 1992).

In group N (ages 35-44 years) the prevalence of infection in 2006 was 11.8%. In subjects in this age group in 1992 (not the same individuals), 24.2% had *O. viverrini* infection. This decrease of 12.4% was statistically significant (95%CI 3.9%-20.9%, p <0.01).

The analysis of factors associated with change in *O. viverrini* infection between 1992 and 2006 is based on subjects in groups 1 and 2 (examined in both 1992 as well as 2006), and is based on faecal egg counts as well as anti-*O. viverrini* antibody titres.

Factors related to change in O. viverrini infection based on stool examination

Table 1 shows that 13 subjects who had been *O. viverrini* negative in 1992 had become *O. viverrini* positive by 2006 (8 in group 1, 5 in group 2).

90% of subjects interviewed in 2006 reported being aware of health promotion campaigns, but this was unrelated to acquisition of infection (crude OR=1.6, 95%CI 0.2-13.7). 69% of subjects reported using praziquantel between 1992 and 2006, but there was no association with becoming positive for OV infection (OR 1.8 95%CI 0.5-6.1). However, acquisition of infection was related to smoking, alcohol use and past infection with *O. viverrini*. Multivariate logistic regression analysis showed that smokers had an odds ratio of 4.6 (95%CI 1.2-16.8) compared to non-smokers, for acquiring *O. viverrini* infection, after adjusting for drinking and previous *O. viverrini* infection. Investigating smoking and drinking habits further, we found strong associations between persistent smoking (smoked in both 1992 and 2006) and frequent alcohol consumption (drinking >15x per month) with the acquisition of *O. viverrini* infection (Table 2). The adjusted OR for persistent smokers in acquiring *O. viverrini* infection was 10.1 (p=0.001) and for frequent alcohol consumption was 5.3 (p=0.026). There was no evidence of interaction between smoking and drinking.

In subjects interviewed in both 1992 and 2006, there

Table 1. Number of Subjects with Opisthorchis Viverrini Infection in 1992 and 2006 in group 1 and Group 2 Subjects

	O. viverrini status in 2006 (group 1)		
	Positive	Negative	Total
*O. viverrini*status in 1992 (Group 1)			
Positive	1	32	33
Negative	8	98	106
Total	9	130	139
*Exact McNemar's chi2 = 14.40, p-value = 0.0002			

	O. viverrini status in 2006 (group 2)		
	Positive	Negative	Total
O. viverrini status in 1992 (Group 2)			
Positive	4	24	28
Negative	5	85	90
Total	9	109	118
*Exact McNemar's chi2 = 12.45, p-value = 0.0005			

Table 2. Past Opisthorchis Viverrini Infection, Persistent Smoking and Frequent Alcohol Consumption with their Adjusted Odds Ratios for Association in Cohort Subjects Who were *O. viverrini* Negative in the 1992 and became *O. viverrini* Positive in 2006.

Characteristics	Number of subjects who were O. Viverrini negative in 1992 and became O. Viverrini positive in 2006	Crude odds ratios	95% confidence interval for crude odds ratios (p-value)	Adjusted odds ratios*	95% confidence interval for adjusted odds ratios (p-value)
Past O. Viverrini infection					
No	6	1		1	
Yes	7	2.6	0.8-8.0 (0.107)	1	0.2-4.8 (0.955)
Persistent smoker					
No	4	1		1	
Yes	6	10.7	2.8-41.6 (0.001)	10.1	2.4-41.6 (0.001)
Alcohol consumption					
Occasional or non-drinkers	4	1		1	
Frequent drinkers	9	7.1	2.1-24.0 (0.002)	5.3	1.2-23.0 (0.026)

*Adjustments were made for all variables in the table

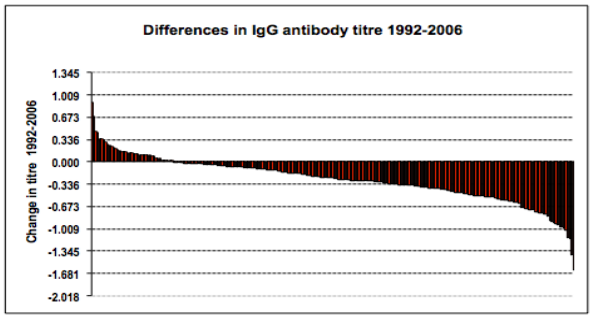

Figure 2. The Differences in IgG Anti-Opisthorchis Viverrini Antibody Titre in Cohort Subjects between 1992 and 2006

had been no significant change in smoking prevalence (21.6% in 2006, with, of those who smoked, 80% being "persistent smokers" (i.e., those who smoked in both 1992 and 2006)). However, there had been a significant increase in alcohol consumption (23.6% drinkers in 1992, 37.9% in 2006 (p-value for the difference = 0.008)).

Factors related to change in O. viverrini infection based on antibody titres

Faecal egg counts provide information only on *O. viverrini* infection status at the moment of testing. Antibody titres may provide additional information on the intensity and duration of past infection, at the time of measurement. Mean antibody titres in cohort subjects were 0.52 (SD 0.34) in 1992 and 0.25 (SD 0.15) in 2006. Figure 2 shows the distribution of the differences in titre for the 241 subjects with paired measurements in 1992 and 2006.

The mean difference in titre was -0.264 (standard error of the difference 0.022). Six out of 241 subjects (2.5%) had an increase in titre ≥1 standard deviation of the mean difference (0.336) above zero change, while 90 had a decrease of the same magnitude below zero. In 145 subjects, the change was less than this.

None of the demographic or behavioural factors were significantly related to change in titre, except for subjects who both smoked and drank in 2006, for whom the change in antibody titre was rather less than in non-smokers and drinkers (mean change -0.09, p=0.07).

Discussion

Our study suggests that the prevalence of *O. viverrini* infection has dropped; in the same individuals, studied after a 14-year interval, *O. viverrini* prevalence in 2006 was 7.0% in contrast to 23.7% in 1992. *O. viverrini* positivity in the whole study group (ages 35-68) was 9.3%, comparable to recently published national prevalence in adults estimates of 9.4% in 2000 and 8.7% in 2009, as well as the 14.2% for Khon Kaen province in 2009 (Jongsuksuntigul et al., 2003; Sithithaworn et al., 2012). Although national rates have been falling, significant heterogeneity exists within Thailand, and some studies have shown little change in north-eastern populations of Thailand: 16.6% in 2009 compared to 15.7% in 2000 (Shin et al., 2010; Sithithaworn et al., 2012). This is thought to be mainly due to the prevalence of aquaculture in the region, the ingrained socio-cultural norms of the population, readily available praziquantel coupled with high re-infection rates in the context of current liver fluke control programmes (Grundy-Warr et al., 2012; Sithithaworn et al., 2012).

Smoking prevalence remains high amongst males whilst females invariably do not smoke. Alcohol consumption increased in cohort subjects between 1992 and 2006. This has important clinical implications: not only has alcohol consumption been found to be associated with an increased risk of CCA (Honjo et al., 2005; Songserm et al., 2012), there has been a recent study which shows that alcohol enhances excystation of O.viverrini metacercariae (Sriraj et al., 2013). This finding is especially important because it is a common misunderstanding in the region that alcohol helps eradicate *O. viverrini* when consumed while eating raw fish (Sriraj et al., 2013).

Although there was a high level of awareness of *O. viverrini* health promotion campaigns in all the subjects (around 90.0%), up to 20.8% had no knowledge of *O. viverrini* and its detrimental health effects. Furthermore, we found no association between exposure to health promotion initiatives and the acquisition of *O. viverrini* infection. As health promotion methods experienced by subjects were mainly through an established method of direct encounters with healthcare staff, a potential opportunity exists to narrow the above discrepancy through modification of information dissemination and health education methods. Currently, health education methods are seldom described and deemed not to be culturally sensitive (Grundy-Warr et al., 2012; Sithithaworn et al., 2012; Sripa et al., 2014). Use of praziquantel is difficult to study by recall, with inevitable misclassification bias. Moreover, because the drug is widely available, it is often misused and, as reported in the literature, re-infection rates are very high. The lack of association between reported past use and current *O. viverrini* infection is therefore not surprising.

In relation to the change in *O. viverrini* infection prevalence over time, we found an association between lifestyle habits and *O. viverrini* infection for cohort subjects who tested negative in 1992 and became positive in 2006. Subjects who persistently smoked and frequently consumed alcohol had an increased risk of acquiring *O. viverrini* infection (adjusted OR of 10.1 and 5.3, respectively). As *O. viverrini* serology may give a better assessment of long term *O. viverrini* exposure, we investigated its utility in assessing the change of *O. viverrini* infection prevalence. Surprisingly, cohort subjects who smoke and drink had a greater reduction in antibody titre levels than those who did not. Although there may be reduced immune responses and increased susceptibility to infections amongst smokers and drinkers (Al-Ghamdi 2007; Shang 2011; Wang 2011), it is worth noting that we examined the relative change in antibody titres of subjects in *O. viverrini* endemic areas where most patients are seropositive; small relative changes may be clinically less important.

The use of antibody testing as a test of cure for *O. viverrini* remains contentious, and some authors have found that ELISA tests are sensitive only when titres in pre-treatment sera are high (Thammapalerd et al., 1988,

Johansen et al., 2010).

In conclusion, our study demonstrated a reduction of *O. viverrini* infection prevalence in Chonnabot, Khon Kaen over a 14-year period. However, it highlighted a potential opportunity for further research on health promotion and education methods. Unhealthy lifestyle habits were common with high smoking rates in males and escalating alcohol consumption rates; it is particularly relevant with our study findings linking smoking and alcohol with the acquisition of *O. viverrini* infection. This emphasises the double burden of disease (infectious and non-communicable) that developing countries are facing, where lifestyle habits are in transition.

Persistently high prevalence of infection in other parts of the north-east, problems with re-infection, easy access to praziquantel, prevalent unhealthy lifestyle habits and static CCA rates attest that this zoonosis remains a considerable public health problem that requires on-going investigation.

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