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Associations between consumption of unsafe fish dishes and knowledge about *opisthorchis viverrini* infection in Southern Laos

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Abstract

Infection by the liver fluke, *Opisthorchis viverrini*, poses a serious public health problem in many areas of the Lower Mekong Basin, where there is a powerful cultural habit of eating unsafely prepared fish dishes. High prevalences of the infection are common in the central and southern regions of Lao PDR. Attempts to control infection rates have often relied heavily on health education to improve knowledge about the infection, and the aim of this cross-sectional analytic study was to test the assumed association between this knowledge and the habit of eating the unsafe dishes in a province of Lao PDR where the infection was considered to be endemic. The knowledge of residents randomly selected from a large number of villages in Khammouane Province was assessed using a structured interview questionnaire, and they were asked about their consumption of various unsafe fish dishes during the past year. Associations between the reported eating of these dishes and knowledge about infection by *Opisthorchis viverrini* were analysed using logistic regression. Apart from knowing that eating uncooked fish with alcohol does not prevent infection, none of the knowledge items was found to be associated with the consumption of the unsafe dishes, and the overall level of knowledge was also unrelated. There has been a dearth of studies on this important issue, especially in Lao PDR, and further research is necessary. However, if the finding is replicated, serious doubts should be raised about the rationale of the current education programmes, and an alternative educational strategy is suggested.

Keywords: *Opisthorchis viverrini; knowledge; unsafe fish dishes; Laos; health education*

1.Introduction

Intestinal helminthiases and fluke infections are the most common neglected tropical diseases in Southeast Asia where there are an estimated 8.03 million cases of opisthorchiasis, mostly in Lao PDR and Thailand[1]. Infection by the liver fluke, *Opisthorchis viverrini (OV)*, is a particularly serious public health problem in the Lower Mekong Basin. While 90-95%of those infected appear to experience no symptoms[2], the fluke is a Group 1 carcinogen[3] and is a major risk factor for the eventual development of a bile duct malignancy, cholangiocarcinoma (CCA), which is almost always fatal due to typically late diagnosis [4,5,6,7,8].

The principal sources of infection by OV are freshwater cyprinid fish, which are one of the two intermediate hosts of the parasite. In the life cycle of OV, the primary hosts are man and various animals, especially cats[9], which serve as reservoir hosts. The primary hosts shed OV eggs in faeces, and the microscopic detection of the eggs in stool samples has been a common basis for the routine diagnosis of an OV infection. The eggs are ingested by a *Bythinia* snail (first intermediate host) and are then encysted and released into water as free-swimming cercariae. These enter the tissues of cyprinid fish (second intermediate host) and develop into metacercariae which can become adult liver flukes in the biliary system of the definitive or reservoir host which consumes the infected

fish[10,11].While a chronic OV infection by adult flukes in the bile ducts of humans can lead to CCA, this process seems to take many years, and the exact pathogenesis is not known, but it is almost certainly multifactorial: the fluke does appear to cause progressive damage to bile duct epithelium and secrete proteins which affect host cell proliferation[12,13,14].

High prevalences of OV infection have occurred and continue to arise in the Lower Mekong basin where freshwater fish have been an important source of food for centuries, and there is strong culturally engrained dietary habit of eating fish dishes and sauces which are likely to contain viable OV metacercariae[15]. These unsafe dishes and sauces are made using freshwater fish ingredients which are raw, undercooked or improperly fermented. In Lao PDR high prevalences of OV infection are especially common in the areas of rice–based cultures in the lowland wetlands of the central and southern regions where local infection prevalence rates of over 90% have been found[16,17,18,19,20,21].

Control strategies have depended mainly on top-down health education programmes and the treatment of infected cases with the anthelmintic drug, praziquantel, and the health education approach has relied heavily on the assumption that increased knowledge about OV infection will lead to a reduction in the consumption of unsafe fish dishes[19]. The aim of the present study was to test the validity of this assumption by exploring the association between knowledge about OV infection and the eating of unsafely prepared freshwater fish dishes in a Lao population which is likely to be at high risk of infection.

2. Materials and Methods

2.1 Study Design, Subjects and Data Collection

The data for this cross-sectional analytic study were collected for a previous study of the prevalence and factors associated with OV infection in Khammouane Province, Lao PDR. This study was conducted over the period June-July, 2015, and basic information about the methodology has already been reported[22].

To summarise and to add further details, 237 subjects were randomly selected from 150 villages in the Thakek District of Khammouane Province. Thakek has population of 88,229 and is an area adjacent to the Mekong River in central Laos which is crossed by many small streams and rivers. The ethnic population is predominantly Lao Lum, who are notorious for their engrained cultural habit of eating raw fish. A requited sample size of 237 subjects was estimated using the appropriate formulae for proportion estimation[23] and multiple logistic regression[24], and a two-stage sampling procedure was used. In the first stage, 10 of all the 150 villages were selected by simple random sampling. The total population of these 10 villages was 3,494. The final sample of 237 participants was then selected using systematic random sampling. The structured questionnaire used to interview the subjects contained 16 items designed to test knowledge about OV infection and its possible consequences. There were three response options for each item ('agree', 'not agree' and 'don't know'), and responses were scored as 'correct ' (1) or 'incorrect' (zero). For seven of the items the correct response was 'not agree', and all 'don't know' responses were recorded as incorrect. The content validity of the questionnaire was checked by three experts in the field who agreed that the items accurately reflected the important aspects of the topic.

The independent variables were age, gender, educational level, occupation, history of praziquantel use, scores on each of the 16 knowledge items, and overall knowledge. To assess their level of overall knowledge subjects were categorised as having higher or lower knowledge as determined by whether their total knowledge score were above or below the mean knowledge score of all the subjects: those obtaining scores equal to or greater than the mean were classified as belonging to the higher knowledge group. The dependent variable was whether subjects reported consuming any one or more of three cyprinid fish sauces, *goi plaa dip*, *laap plaa dip* and *som plaa dip*: eating one or more of these fish sauces was coded as 1 and not eating any of them was coded as zero. Although subjects had originally been asked about the consumption of five different types of fish sauce, these three sauces all contained raw fish and were considered to be the most likely to be infected with viable OV metacercariae.

2.2 Data Analysis and Ethical Approval

The data were summarised with descriptive statistics, and unconditional logistic regression was used to analyse factors associated with the consumption of unsafe fish dishes. Those factors significant at $p \leq 0.05$ in the univariate analysis were included as candidate variables in the multivariate analysis using backward elimination. Statistical significance in the multivariate model was set at $p \leq 0.05$. All analyses were performed using Stata version 10.0[25].

The study was approved by the KhonKaen University Ethics Committee for Human Research (reference no. HE582126)

3. Results

Table 1 shows the number and percentages of subjects who reported consuming one or more of the three unsafe fish sauces in the past year. A large majority (89.5%) said they had eaten at least one of the unsafe dishes and over half (52.3%) had eaten all three sauces.

Summary characteristics of the sample are included in Table 2. A slight majority were male (52.7%), and most were 35 years of age or older (68.4%). A majority were also educated above primary school level (57.0%) and were employed in work not directly associated with farming (62.9%). Most (79.7%) reported no history of taking the anthelmintic drug, praziquantel.

The outcomes of the logistic regression analyses are also shown in Table 2. In the final model, the only independent variables associated with the consumption of unsafe fish dishes were gender and knowledge about the potential protective effect of alcohol. Males were more likely than females to have consumed an unsafe fish sauce (OR_{adj} 2.37, 95%CI 1.39-4.04), and those who correctly disagreed with the statement that eating uncooked fish with alcohol kills flukes were less likely to have consumed an unsafe fish sauce than those who agreed with it (OR_{adj} 0.53, 95%CI 0.30-0.95).

Table 1 Number and percentage of subjects consuming unsafe sauces in past year

Unsafe sauces	Consumed		Not consumed	
	n	%	n	%
1 type of sauce only				
Goi plaa dip	176	74.26	61	25.74
Laap plaa dip	181	76.37	56	23.63
Som plaa dip	162	68.35	75	31.65
2 types of sauce				
Goi plaa dip and laap plaa dip	160	67.51	77	32.49
Goi plaa dip and som plaa dip	133	56.12	104	43.88
Laap plaa dip and som plaa dip	138	58.23	99	41.77
All 3 types of sauce	124	52.32	113	47.68
At least 1 or more types of sauce	212	89.45	25	10.55

Table 2 Summary of characteristics and knowledge of subjects and results of logistic regression analyses

Factors	Subjects		% ate	Crude OR	95% CI	p-value	Adj. OR	95% CI_{adj}	$p\text{-value}_{adj}$
	number	percent							
1. gender						<0.001			0.002
- female	112	47.3	40.2	1			1		
- male	125	52.7	63.2	2.56	1.51 - 4.32		2.37	1.39 - 4.04	
2. age (years)						0.729			
< 35	75	31.7	50.7	1					
36 or older	162	68.4	53.1	1.10	0.64 - 1.91				
3. education						0.923			
- above primary	135	57.0	52.6	1					
- primary	102	43.0	52.0	0.97	0.58 - 1.63				
4. occupation						0.870			
- other	146	61.6	52.7	1					
- faming	91	38.4	51.7	0.96	0.57 - 1.62				
5. praziquantel						0.719			
- never taken	189	79.8	52.9	1					
- taken	48	20.3	50.0	0.89	0.46 - 1.68				
6. OV infection is caused by eating uncooked or semi-cooked cyprinoid fish						0.747			
- incorrect	61	25.7	54.1	1					
- correct	176	74.3	51.7	0.91	0.51 - 1.63				
7. There is a risk of OV infection from eating cyprinoid fish						0.446			
- incorrect	74	31.2	48.6	1					
- correct	163	68.8	54.0	1.24	0.77 - 2.15				

Factors	Subjects		% ate	Crude OR	95% CI	p-value	Adj. OR	95% CI_{adj}	$p\text{-value}_{adj}$
	number	percent							
8. OV eggs in humans are excreted in stools						0.678			
- incorrect	104	43.9	53.8	1					
- correct	133	56.1	51.1	0.90	0.54 - 1.50				
9. Distension, flatulence, diarrhea, anorexia, sore right ribcage and jaundice are symptoms of liver fluke infection						0.029			
- incorrect	173	73.0	56.7	1					
- correct	64	27.0	40.6	0.52	0.29 - 0.94				
10. Stool examination is a method for checking the presence OV eggs						0.937			
- incorrect	54	22.8	51.8	1					
- correct	183	77.2	52.5	1.02	0.56 - 1.88				
11. An OV infected person can be cured						0.820			
- incorrect	53	22.4	50.9	1					
- correct	184	77.6	52.7	1.07	0.58 - 1.98				
12. An OV infected person can have re-infection after being cured						0.058			
- incorrect	64	27.0	53.8	1					
- correct	173	73.0	50.9	0.57	0.31 - 1.02				
13. Defaecation in toilet is a way to prevent OV spread to other people						0.948			
- incorrect	52	21.9	51.92	1					
- correct	185	78.1	52.43	1.02	0.55 - 1.88				
14. One way of preventing the spreading of OV infection to other people is to ensure that the disposal of the waste products of food containing uncooked fish is done in a correctly and hygienically so that cats and dogs are not allowed to eat the waste food						0.651			
- incorrect	119	50.2	53.8	1					
- correct	118	49.8	50.8	0.89	0.53 - 1.48				
15. A healthy person will not get an OV infection						0.811			
- incorrect	182	76.8	52.8	1					
- correct	55	23.2	50.9	0.93	0.51 - 1.70				

Factors	Subjects		% ate	Crude OR	95% CI	p-value	Adj. OR	95% CI_{adj}	$p\text{-value}_{adj}$
	number	percent							
16. You will have a stool test examination to find OV infection only when you are sick						0.033			0.080
- incorrect	120	50.6	59.2	1			1		
- correct	117	49.4	45.3	0.57	0.34 - 0.95		0.62	0.36 - 1.05	
17. If we eat uncooked fish only rarely, we will not get an OV infection						0.139			
- incorrect	152	64.1	55.9	1					
- correct	85	35.9	45.9	0.67	0.39 - 1.14				
18. Eating uncooked fish with alcohol will kill the flukes						0.007			0.032
- incorrect	84	35.4	64.3	1			1		
- correct	153	64.6	45.8	0.47	0.28 - 0.81		0.53	0.30 - 0.95	
19. The flukes will die if you add lemon juice to raw fish						0.751			
- incorrect	99	41.8	53.5	1					
- correct	138	58.2	51.5	0.92	0.55 - 1.52				
20. You will not get an OV infection from eating Plaa som or Plaa jom raw because the flukes have been killed						0.904			
- incorrect	106	44.7	51.9	1					
- correct	131	55.3	52.7	1.03	0.62 - 1.72				
21. The medicine for killing flukes is easy to get and is free, so there is no need to stop eating raw fish						0.374			
- incorrect	93	39.2	55.9	1					
- correct	144	60.8	50.0	0.79	0.47 - 1.33				
22. correct knowledge score						0.651			
- high knowledge score (>9)	118	49.8	50.9	1					
- low knowledge score (<= 9)	119	50.2	53.8	1.12	0.68 - 1.87				

Hosmer-Lemeshow goodness-of-fit test = 0.481

4. Discussion and Conclusions

The purpose of this study was to investigate the association between knowledge about OV infection and the reported consumption of fish sauces which were likely to contain viable metacercariae. The results showed that, with one exception, none of the OV knowledge factors were associated with the consumption of these sauces, including the overall level of knowledge (higher vs. lower).

This finding is at variance with the outcome of a cross-sectional study conducted among villagers in Northeast Thailand, which found that the participants' awareness of the health risks of OV infection was negatively related to their reported eating of raw fish dishes: the odds of a villager reporting the avoidance of these dishes were eight times higher in those aware of the infection risk than in those who were not[26]. To our knowledge, there have been no other studies of this kind in the regions of Southeast Asia where OV infection is endemic, and, without these, any explanation for the inconsistent findings must be highly speculative. However, it is possible that the cultural pressures to eat unsafely prepared fish dishes in the study area were stronger and more difficult to override than in the Thai villages. A study of villagers in Saravane Province in southern Laos highlights how deeply engrained in the local culture is the habit of eating unsafe fish dishes with strong pressures to serve these dishes at festivals and at social and religious events where refusal to eat them would be met with social disapproval[27]. The cultural strength of the habit is reflected in the very high rates of consumption of raw or unsafely prepared fish reported in southern Laos villages, rates of 80% or higher among almost half 13 villages surveyed in Saravane Province[18] and almost 90% in the present study.

The finding that men were more likely to eat the unsafe fish dishes was also reported in the Saravane study[27] and in the Northeast Thailand study[26]. The likely explanation for this is the one offered in both these studies and is that the habit of eating unsafe fish dishes appears to have a strong cultural association with social gatherings of males. It is therefore not surprising that male gender is commonly associated with higher OV infection rates whereas female gender is not[22, 28].

The one exception to the lack of association between OV knowledge and the eating of unsafe fish sauces was the protective factor of knowing that eating uncooked fish with alcohol does not prevent infection. There seems to have been strong cultural practice of drinking alcohol when eating raw fish, and this finding is in contrast with the apparently popular belief, at least among the frequent primarily male informal gatherings of fishermen along the banks of the Mekong, that the consumption of alcohol kills parasites[15].

The lack of association between knowledge about OV infections and the consumption of unsafe fish dishes is indirectly supported by a study which found no relationship between knowledge and infection status in Mahasarakham Province[28]. While the main finding of the present study is not supported by the Northeast Thailand study cited above[26], there is a lack research on this issue which has important implications for health education campaigns based on the assumption that people will avoid eating unsafe dishes if they are well informed about OV infection and the serious associated health risks. One important limitation of the present study is its failure to ask respondents about the frequency with which they consumed unsafe sauces.

Clearly, further studies are needed and, if the present general finding is confirmed, a more sophisticated educational approach is needed which directly confronts the common apparent paradox of people knowingly willing to take serious risks with their lives. An example of such an approach is one advocated by Fotuhi and colleagues[29] in relation to smoking cessation and would involve identifying the specific risk-minimising and functional beliefs which those consuming unsafe dishes are adopting to justify their behaviour. An educational programme would then be designed which specifically counters these beliefs.

The consumption of what the public health community regards as unsafe fish dishes is a cultural practice which was been deeply embedded in the Mekong region over many generations and has become an important part of the cultural identity of the people who live there and are bonded together by it. The complexity and power of the socio-ecological factors underlying what is now seen as a public health problem suggests that a transdisciplinary approach is needed to foster a better understanding these factors if health education programmes are to succeed[30,31].

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