RISK FACTORS FOR *OPISTHORCHIS VIVERRINI* INFECTION AMONG SCHOOLCHILDREN IN LAO PDR

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Abstract. A matched-pair case-control study was conducted to identify factors associated with *Opisthorchis viverrini* infection among primary schoolchildren to develop preventive education. A house-to-house interview of the guardians of 118 children (59 cases and 59 controls) was conducted to collect information about theirs and their children's fish eating habits of 10 locally available fish species. The guardians' knowledge and attitudes about *Opisthorchis viverrini* infection and socio-economic status were asked about. The frequencies of eating fish did not differ between cases and controls. However, cases ate raw fish more frequently than controls (5.1 *vs* 1.2 times monthly). The frequency of eating raw "pa-xiew" was most strongly related to infection (OR 2.47; 95%CI 1.05-5.82). Infection was significantly associated with the children's experiences of eating raw fish (OR 7.48; 95% CI 1.45-38.69), frequency of eating raw fish by their guardians (OR 1.26; 95% CI 1.04-1.53) and maternal educational and career (OR 0.76; 95% CI 0.62-0.94). To prevent infections it is necessary to prevent the fish from becoming infected and to avoid eating raw fish. Education should focus on the children and their guardians to promote better eating habits.

Keyword: *Opisthorchis viverrini*, risk factors, schoolchildren, Lao PDR

INTRODUCTION

Infection with *Opisthorchis viverrini* (Ov), a food-borne trematode known as "Thai liver fluke", is an endemic public health problem in Lao PDR and northeastern Thailand (WHO, 1995; Kobayashi and Sato, 2000; Kobayashi *et al*, 2000; Rim *et al*, 2003; Sithuthaworn and Haswell-Elkins, 2003; Chai *et al*, 2005). Approximately a third of the population of Lao PDR, or 2 million people, are infected (WHO, 1995). More than a half of adults are infected in endemic areas of Lao PDR (Pholsena *et al*,

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1991; Kaneda *et al*, 2006; Sayasone *et al*, 2007). Although early stages of infection are less serious, the severity increases with duration and intensity of infection, and is associated with cholangitis, obstructive jaundice, hepatomegaly, cholecystitis, cholelithiasis and cholangiocarcinoma (Elkins *et al*,1990; Sithithaworn *et al*, 1994; Mairiang and Mairiang, 2003).

There are two intermediate hosts in the lifecycle of Ov: the *Bithynia* snail and cyprinid fish (Wykoff *et al*, 1965; Harinasuta and Harinasuta, 1984). Humans become infected by eating raw or undercooked cyprinid fish (Migasena, 1982; Harinasuta and Harinasuta, 1984; Kurathong *et al*, 1987; Sithuthaworn and Haswell-Elkins, 2003; Sayasone *et al*, 2007). The high prevalence of infection is also related to poor toilet facilities (Sayasone *et al*, 2007). Health education to prevent or to reduce raw fish consumption, de-worming with praziquantel, mass drug administration, and improvements in hygiene are methods to reduce prevalence and intensity of infection (Wykoff *et al*, 1965; Harinasuta and Harinasuta, 1984). The prevalence of infection among primary schoolchildren reaches 60% in endemic areas (Kaneda *et al*, 2006b). Eating raw fish during early childhood is associated with a high prevalence of Ov infection among children (Shinzato *et al*, 2004). There are no reports on consumption of raw fish by schoolchildren in endemic areas. Poor economic situation and education have been found to be associated with intestinal helminth infections (Phiri *et al*, 2000; Traub *et al*, 2004; Raso *et al*, 2005; Wördemann *et al*, 2006; Quihui *et al*, 2006; Raso *et al*, 2006; Matthys *et al*, 2007). This association has never been reported with Ov infection; therefore, careful sociological investigation is needed (Migasena, 1982; WHO, 1995). The association between Ov infection and fish-eating habits and methods of fish preparation needs further investigation (Kobayashi and Sato, 2000). According to an unpublished study we conducted in Lao PDR, over 90% of households catch fish themselves, from various places, such as rivers, ponds, irrigation canals and wet rice fields. The fish is cooked by various methods depending on the species, size, number of fish, and the time and labor available for preparation. There is no information regarding the kinds of cyprinid fish which are frequently eaten raw and the relationship between cyprinid fish eating habits and Ov infection.

School health services in Lao PDR have developed rapidly in recent years, and include supplying teaching materials, de-worming, and constructing latrines**.** However, health education about prevention and control of Ov infection is poorly implemented; there are inadequate textbooks and teaching materials (Ministry of Education, Lao PDR, 1997). The absence of information about factors related to Ov infection among children is a barrier to effective Ov control. Inadequate school health education regarding control of Ov infection results in inadequate knowledge among students, teachers and health personal. In order to develop a school health education program for prevention and control of Ov in Lao PDR as a long-term strategy, a case-control study of Ov infection was conducted among schoolchildren in an endemic area. Thus, the present study aimed to identify factors related to Ov infection in primary schoolchildren in Lao PDR. Fish species eaten raw and risk factors for infection by the fish species were also investigated.

MATERIALS AND METHODS

Study area and subjects

The study was conducted in the Lahanam area of Songkhone District, Savannakhet Province, Lao PDR. The area is along the Xe Banghiang River, a tributary of the Mekong River. According to a census conducted in 2005, the area consists of six villages with 713 households and 4,413 residents. A demographic surveillance system with monthly household visits was established in this area in 2004. Most villagers engage in wet rice farming, and fishing in the Xe Banghiang River, small streams, ponds and wet rice fields (Kanada *et al*, 2006a).

The case-control study was conducted as follows. Stool samples from 730 children at 5 primary schools were examined by Kato-Katz method (Elkins *et al*, 1990). The prevalence of Ov infection was 63%. Seventy-four children had Ov eggs at a concentration of > 1,000 eggs per gram (epg) of stool. All Ov egg-positive children were treated with praziquantel and albendazole. Stool examinations were repeated one week later and controls were selected from the 204 children who were Ov egg-negative or both examinations. Among these, 74 controls were randomly selected, matching for school, sex and age (±one year); finally, 61 pairs were chosen. Interviews with guardians were conducted in house-to-house visits using a semi-structured questionnaire. Interviewers were blinded regarding infection status until all field activities were completed.

Questionnaire development

Preliminary study to identify cooking methods and fish species. Eight focus group discussions were conducted prior to the study to develop a questionnaire. The methods of preparing fish and information regarding fish eaten were collected prior to the survey. Three types of food preparation were identified: 1) raw, 2) insufficiently-cooked and 3) well-cooked. Raw fish meals were *Koi-pa dip* (chopped raw fish), *Laap-pa dip* (minced raw fish with water/fish paste), and *Cham-pa dip* (chopped raw fish eaten with chilly sauce). Insufficiently-cooked fish were *Laap-sa pa* or *Laap-sut (soy*), which were parboiled in water at 50-80ºC for approximately 30 seconds. Well-cooked fish meal was called *Koi- pa souck* (cooked *Koi-pa dip)*, *Laap-pa souck* (cooked *Laap-pa dip*), *Cham-pa souck* (cooked *Cham-pa dip*), *Ping-pa* (grilled fish), *Keng-pa* (fish soup with vegetables), *Chum-pa* (fried fish), *Mok-pa* (steamed fish with banana leaf), *Or-pa* (boiled and crushed fish) and *Tom-pa* (boiled fish). The present study focused on raw fish meals.

The names of fish eaten locally were obtained during the focus group discussions; 160 fish names were listed. Among them, 46 species were previously reported to have Ov metacercaria (Wykoff *et al*, 1965;Vichasri *et al*, 1982; Harinasuta and Harinasuta, 1984; Giboda *et al*,1991a,b; Scholz *et al*, 1991; Sitithaworn *et al*, 1997; Srisawangwong *et al*, 1997; Waikagul, 1998; Sukontason *et al*, 1999; Nithiuthai *et al*, 2002; Sayasone *et al*, 2007); 20 species were frequently eaten and 27 species were frequently eaten raw. Ten species most frequently and eaten raw were selected for the questionnaire. Eight were previously reported as being infected with Ov metacercaria (Lao local name in parentheses): *Puntioplites falcifer* (Pa-sakang), *Hypsibarbus pierrei* (Pa-park), *Osteochilus hasselti*, (Pa-eh thai), *Mystacoleucus atridorsalis* (Palang nam), *Puntius brevis* (Pa-khao mon), *Hampala macrolepidota* (Pa-sout), *Esomus metallicus* (Pa-xiew), *Cyclocheilichthys enoplos* (Pa-chork), (*Labeo chrysophekadion* (Pa-phiya) and *Oreochromis niloticus* (Pa-

ning). The final 2 were never reported to have metacercaria infection. A picture book was used to identify fish species and local fish names (Baird, 1999).

Questionnaire

The questionnaire consisted of questions about: 1) fish eating habits, 2) knowledge and attitudes of guardians regarding Ov infection, 3) attitudes of guardians about their children's raw fish eating habits, and 4) demographic and socio-economic factors. Fish eating habits, preferences, experiences, frequencies of eating fish and raw fish were enquired about. Eating habits regarding 10 locally available fish species frequently eaten and commonly eaten raw were asked about among children and guardians. The children's ages at onset of eating raw fish were asked about. Knowledge and attitudes of guardians about Ov infection, causes, symptoms, treatment, methods of prevention, and their experiences with Ov infection were asked about. Attitudes of guardians about their children's raw fish eating habits, if they ever heard/believed a child could get sick from eating raw fish and if they ever heard/believed giving raw fish to a child is taboo were asked about. For demographic and socio-economic factors, data about the number of household members, number of male household members, availability of fish (number of household members who go fishing, frequency of fishing by household members), maternal education, possession of assets, such as a car, motorbike, bicycle, cultivator, television, radio, electric fan, mobile phone, refrigerator, livestock (chicken, pig, buffalo, duck, goat, cow), hygiene facilities(toilet) or wet rice field, were asked about.

Data management and statistical analysis

The frequencies of eating fish/raw fish were calculated per month. With regard to preferences in eating fish / raw fish those who answered "cannot answer because of no experience of intake" were excluded from the study. Eight fish have been reported to be infected with Ov metacercaria and classified by habitat (Iwata *et al*, 2003; Poulsen *et al*, 2004). The fish were classified in four groups: 1) river fish (Pa-sakang, Pa-park, Pa-lang nam, Pa-chork), 2) pond fish (Pa-sout) 3) irrigation canal fish (Pa-eh thai, Pa-khao mon), 4) rice field fish (Pa-xiew). The association of between Ov infection and consumption of fish, attitudes of guardians regarding Ov infection, their children's raw fish consumption, demographic and socio-economic factors were tested by the Mc Nemar test or Wilcoxon matched-pairs signed-ranks test. The risk of Ov infection was evaluated by odds ratio (OR) and 95% confidence interval (95% CI). Risk factors related to Ov infection were evaluated by conditional logistic regression analysis (forward selection method: likelihood ratio). The level of statistical significance was set at $p<0.05$. All data was entered and double-checked by two individuals using Microsoft Excel 2003. Data were analyzed using the statistical software package SPSS, Version 12.0.

Ethical considerations

This study was approved by the ethics committee of Hiroshima University (No.158) and the National Ethics Committee for Health Research, Lao PDR (No. 172). Informed consent was obtained from each guardian verbally. Before inclusion in the study we explained the aims, procedures, and potential risks and benefits of this study to the guardians in the Lao language, and informed the guardians participation was voluntary and could be stopped at any time.

Table 1
Demographic and socio-economic characteristics of cases and controls.

Demographic and socio-economic characteristics	Cases (n=59)	Controls (n=59)	p-value
	Mean (SD)		
Number of household members	8.2 (3.1)	7.3 (3.2)	0.042
Number of male household members	4.1 (2.3)	3.4 (1.9)	0.042
Number of household members who go to fishing	2.4 (0.9)	1.9 (1.0)	0.006
Mother's education careers (years in school)	3.0 (3.1)	5.0 (4.0)	0.003
Wet rice field (ha)	2.4 (2.0)	2.1 (2.0)	0.070
	% Households owning at least one		
Car	1.7	0.0	1.000
Motorbike	40.7	66.1	0.012
Bicycle	67.8	69.5	1.000
Cultivator	44.1	52.5	0.472
Television	69.5	81.4	0.210
Radio	66.1	54.2	0.248
Electric fan	71.2	84.7	0.115
Mobile phone	28.8	47.5	0.072
Refrigerator	20.3	42.4	0.021
Latrine	30.5	44.1	1.000
	% Households raising at least one		
Chicken	74.6	74.6	1.000
Pig	35.6	35.6	1.000
Buffalo	44.1	33.9	0.380
Duck	57.6	52.5	0.750
Goat	27.1	20.3	0.500
Cow	62.7	57.6	0.700

McNemar's test and Wilcoxon matched-pairs signed-rank test

RESULTS

Of the 61 matched pairs, information was collected for 59 pairs (96.7%, 29 pairs of boys and 30 pairs of girls; total 118 children). The ages of the subjects ranged from 5 to 16 years (mean; 9.8 years old). The median concentration of Ov eggs was 2,550 epg of stool. The guardians who answered the questionnaires were 54 mothers and 5 grandmothers for the cases, and 56 mothers, 2 grandmothers and one father for the controls.

The demographic and socio-economic factors among cases and controls are shown in Table 1. The number of household members, especially male members and those who went fishing were more in the cases than in controls. Among controls the maternal education level was higher than cases and they owned more assets, such as refrigerators and motorbikes. There were no significant differences in other demographic or socio-economic factors between cases and controls.

Table 2
Experience, frequency and preference of eating fish/raw fish for cases and controls.

	Cases (*n*=59)	Controls (*n*=59)	*p*-value	Guardian of cases (*n*=59)	Guardian of controls (*n*=59)	*p*-value
Experience of eating fish	100%	100%	NA	100%	100%	NA
Experience of eating raw fish	71.2%	23.7%	<0.001	89.8%	83.1%	0.454
Experience of eating raw fish before entering school	44.1%	13.6%	<0.001	ND	ND	NA
Frequency of eating fish (days per month with SD)	19.5(6.8)	17.5(6.9)	0.160	19.4(6.8)	17.5(6.9)	0.157
Frequency of eating raw fish (days per month with SD)	5.1(7.1)	1.2(2.7)	<0.001	6.9(7.4)	3.1(3.6)	<0.001
Preference of eating fish	94.9%	94.9%	1.000	98.3%	93.2%	0.180
Preference of eating raw fish	48.8.%	28.6%	0.083	49.2%	22.0%	0.004

McNemar's test and Wilcoxon matched-pairs signed-rank test; NA, not applicable; ND, no data

Factors related to Ov infection among primary schoolchildren

Experience, frequency and preferences for eating fish and raw fish are shown in Table 2 for both children and guardians of cases and controls. There were no significant differences between cases and controls and among guardians regarding fish eating preferences. However, cases and their guardians liked eating raw fish more than controls; cases ate raw fish more than controls (71.2% *vs* 23.7%, $p<0.001$). The rate of children eating raw fish prior to entering primary school (under 6 years old) was 44.1% among cases, which was higher than among controls (10%, $p<0.001$). On average, cases ate raw fish 5.1 days per month, which was higher than controls (1.2 days per month, $p<0.001$). The guardians of cases ate raw fish 6.9 days per month, while the guardians of controls ate raw fish 3.1 days per month ($p<0.001$).

The guardian's knowledge, attitudes and perceptions regarding Ov infection are shown in Table 3. Most guardians had heard of Ov, but guardians who knew about Ov infection were few (30.5% *vs* 25.4%, $p<0.648$). There were no significant differences in guardians' knowledge, attitudes and perceptions about Ov infection and attitudes about giving raw fish to children between cases and controls.

Risk factors for Ov infection by conditional logistic regression analysis

Results of conditional logistic regression analyses are shown in Table 4. Sixteen factors with a significant relationship with OV infection by single regression analysis, along with sex, age and village information, were included in the model. Three factors confirmed with multicollinearity were excluded from the model. An infection was significantly influenced by a history of the child eating raw fish as reported by the guardian (OR 7.48; 95% CI

Table 3
Guardian's knowledge, attitude and perception.

	Proportion of "yes" (%)		
	Guardians of cases (*n*=59)	Guardians of controls (*n*=59)	*p*-value
Ever heard that a child will be sick if one eats raw fish	96.6	94.9	1.000
Believe that a child will be sick if one eats raw fish	96.6	100	0.500
Ever heard that giving raw fish to child is taboo	69.5	81.4	0.167
Believe that giving raw fish to child is taboo	71.2	79.7	0.332
Believe that a child should not eat raw fish	44.1	49.2	0.406
Ever heard of OV	88.1	94.9	0.344
Know about OV	30.5	25.4	0.648
Know about cause of OV	35.6	33.9	1.000
Know about symptom of OV	25.4	18.6	0.481
Know about prevention of OV	35.6	30.5	0.648
Know about treatment of OV	16.9	15.3	1.000
Think that oneself is infected with OV	18.6	16.9	1.000
Think that one's child is infected with OV	18.6	23.7	1.000
Have patients of OV among family or relatives	42.4	50.8	0.170

McNemar's test and Wilcoxon matched-pairs signed-rank test

Table 4
Factors influence to children's Ov infection.

	β	OR	95%CI	*p*-value
Children's experience of eating raw fish	2.03	7.48	1.45 - 38.69	0.016
Frequency of eating raw fish by guardians	0.23	1.26	1.04 - 1.53	0.022
Mother's educational career (year)	-2.70	0.76	0.62 - 0.94	0.012

Conditional logistic analyses

1.45-38.69), frequency of eating raw fish by the guardian (OR 1.26; 95% CI 1.04-1.53) and maternal education (OR 0.76; 95% CI 0.62-0.94).

Species of fish eaten raw and risk of infection by fish species

The frequencies of consumption of the 10 fish species by children and their guardians are shown in Table 5. Cases ate raw fish more than controls for almost all species. The same finding was observed for guardians. Fish species frequently consumed raw by cases were: 1) Pa-sa kang, 2) Pa-phiya, 3) Pa-park, and 4) Pa-xiew. Multiple logistic analysis using frequency of eating raw fish among the 10 species revealed a frequency of eating raw Pa-xiew was most strongly associated with Ov infection (OR 2.47; 95%CI 1.05-5.82)

Table 5
Frequency of eating/raw for each fish species for cases and controls.

Local name (scientific name) (days per month with SD)	Cases (n=59)	Controls (n=59)	p-value
Pa-sakang (*Puntioplites falcifer*)	7.5(7.6)	7.0(6.4)	0.538
Pa-sakang (*Puntioplites falcifer*) raw	3.0(6.0)	0.9(3.0)	0.002
Pa-park (*Hypsibarbus pierrei*)	6.4(7.2)	5.3(6.1)	0.484
Pa-park (*Hypsibarbus pierrei*) raw	2.1(3.9)	0.3(0.9)	<0.001
Pa-eh thai (*Osteochilus hasselti*)	4.5(6.4)	2.1(4.7)	0.010
Pa-eh thai (*Osteochilus hasselti*) raw	0.7(1.4)	0.1(0.4)	0.001
Pa-langnam (*Mystacoleucus atridorsalis*)	4.4(6.9)	3.7(7.2)	0.268
Pa-langnam (*Mystacoleucus atridorsalis*) raw	0.4(1.0)	0.2(0.5)	0.131
Pa-khao mon (*Puntius brevis*)	4.2(6.2)	3.8(6.5)	0.377
Pa-khao mon (*Puntius brevis*) raw	0.3(1.3)	0.1(0.3)	0.120
Pa-sout (*Hampala macrolepidota*)	3.7(4.0)	2.5(3.7)	0.081
Pa-sout (*Hampala macrolepidota*) raw	1.0(2.3)	0.1(0.2)	0.001
Pa-xiew (*Esomus metallicus*)	3.0(4.9)	2.0(4.5)	0.022
Pa-xiew (*Esomus metallicus*) raw	1.3(2.9)	0.1(0.3)	<0.001
Pa-chork (*Cyclocheilichthys enoplos*)	2.2(4.3)	1.5(2.7)	0.080
Pa-chork (*Cyclocheilichthys enoplos*) raw	0.9(2.7)	0.2(1.0)	0.003
Pa-phiya (*Labeo barbatulus*) (infection never reported)	4.4(6.9)	4.3(5.5)	0.480
Pa-phiya (*Labeo barbatulus*) raw	2.4(5.8)	0.6(2.6)	0.003
Pa-ning (*Oreochromis niloticus*) (infection never reported)	2.1(4.0)	1.7(3.1)	0.672
Pa-ning (*Oreochromis niloticus*) raw	0.6(1.5)	0.04(0.3)	0.003
Eating raw fish which are captured from river	5.1(9.2)	2.9(5.0)	0.038
Eating raw fish which are captured from pond	0.7(2.1)	0.3(0.9)	0.245
Eating raw fish which are captured from small river	0.7(1.7)	0.4(1.4)	0.175
Eating raw fish which are captured from rice field	0.5(1.3)	0.2(0.8)	0.115

Wilcoxon matched-pairs signed-rank test

(data not shown). Frequency of eating river fish raw was significantly associated with Ov infection (p=0.038).

DISCUSSION

Previous studies have shown eating raw fish is a risk factor for Ov infection (Migasena, 1982; Harinasuta and Harinasuta, 1984; Kurathong *et al*, 1987; Sithithaworn and Haswell-Elkins, 2003; Sayasone *et al*, 2007). This matched-pair case-control study also showed Ov infection among schoolchildren was related to eating raw fish. Ov infection among children was related to frequency of their guardians' consumption of raw fish and maternal education. However, Ov infection in children was not related to the guardian's knowledge, attitudes or perceptions about

Ov infection or attitudes about giving raw fish to children.

It has been reported eating habits in childhood are strongly influenced by their guardians (Birch and Jennifer, 1995). Raw fish consumption by children was influenced by their guardian's eating habits. Most of the children ate with their guardians even in the research area. These eating habits may have been influenced by raw fish eating habits among children and their guardians in this area.

Previous studies have reported poor maternal education level is a risk factor for intestinal helminth infection (Carneiro *et al*, 2002; Nematian *et al*, 2004; Okyay *et al*, 2004). This study also confirmed poor maternal education level is a risk factor for their child's OV infection. Fishing was more common among household members in cases than controls. There is a greater chance of consuming raw fish in a family who fishes frequently, because of the availability of fresh fish. Cases had fewer assets than controls, which probably mean the cases were from a lower socio-economic level than controls. Although previous studies reported lack of a latrine is related to Ov infection, no such relationship was seen in this study (Sayasone *et al*, 2007).

Cases ate raw Pa-sa kang, Pa-phiya, Pa-park and Pa-xiew frequently. These fish were eaten more than 1 time per month. The frequency of eating raw Pa-xiew and river fish was significantly related to Ov infection. According to a survey conducted in neighboring Salavan Province, metacercaria infection rates were low for Pa-sa kang and Pa-park (Sayasone *et al*, 2007), and metacercariae were never reported from Pa-phiya. Metacercaria infection rates were high with Pa-sout, Pa-chork, Pa-langnom and Pa-khao mon. However, these fish were eaten less than 1 time per month in the present study. These results indicate it is not always the fish with high Ov metacercaria infection rates that are eaten frequently. Poulsen *et al* (2004) reported a large number of *Esmos matallicus* (Pa-xiew) were captured in the research area; this fish species spawns and grows in wet rice fields without recurrent migration. The Bithynia snail, the first Ov intermediate host, also inhabits wet rice fields. Main means of subsistence in Ov endemic areas is agriculture, often planting rice. There are usually no toilet facilities in this situation. Wet rice fields are a convenient place to contract Ov infection where people's feces, Bithynia snails and cyprinid fish are all present. From the data of our unpublished study in Lao PDR, we found people who live in Ov endemic areas prefer eating river fish raw, because they believe these fish are fresh. People prefer eating small fish raw, such as *Esmos matallicus* (Pa-xiew). These peoples' beliefs about raw fish influence their eating habits, contributing to Ov infection.

In order to control Ov infections among children, guardians need more information about Ov infections. Children and their guardians should be targeted for health education because guardians' fish eating habits influence their children's risk for Ov infection. The prevention of Ov infection starts from childhood by cooperation between the community and school health programs. Further studies are needed to clarify factors related to fish consumption among children and their guardians. An Ov prevention program at schools is already present in Lao PDR, but prohibition of eating raw fish is not easy because people like to eat raw fish and freshwater fish is an important source of protein in the Laotian's diet (Iwata *et al*,

2003). As an alternative strategy for Ov control, it may be better to recommend avoiding eating raw high-risk fish (Pa-xiew, Pa-sakang, Pa-park, Pa-lang nam, Pa-chork). Further studies are needed to identify human risk for Ov infection, such as clarification about Ov metacercaria infection among fish species frequently eaten raw in endemic areas.

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