Epidemiology of *Opisthorchis viverrini* infection in a rural district of southern Lao PDR

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**Running Title:** Epidemiology of *O. viverrini* in a rural district of Laos

**Conflict of interest:** none to declare
Abstract

Objective: to describe epidemiological features of *O. viverrini* infection in a highly endemic district.

Methods: A random sample of 13 villages and 15 randomly selected households per village was investigated. All household members older than 6 months were included. Each study participant underwent a short clinical examination and was interviewed on behaviour and nutritional risk factors. A stool sample was examined using Kato-Katz technique. Fish samples in each village were dissected for the presence of meta-cercariae.

Results: 814 persons were enrolled. 51.5% were women. Median age was 16 years. 58.5% were infected with *O. viverrini*. Infection rates were similar between sex but increased by age from 20.0% in children below 6 years to 85.5% in adults between 45 and 55 years of age (p<0.001). Intensity of infection and the habit to consume raw or insufficiently cooked fish increased with age, too. 75.2% of the study participants reported to consume raw or insufficiently cooked fish. Multiple helminth infections were frequent. 23 different species of *cyprinoid* fish are consumed in the study villages of whom 20 species were infected with meta-cercariae. At village level the prevalence of raw fish consumption was strongly associated with infection rate (r=0.76, p=0.003). Only in one village latrines were available in the household. 95.0% of the study participants reported to defecate in the forest. A logistic regression model identified age, the consumption of raw or insufficiently cooked fish and absence of sanitation as main risk factors at individual level.

Conclusion: The study documents highly prevalent for *O. viverrini* infection and transmission indicators. Community interventions are urgently needed.
**Keywords:** opisthorchiasis, *Opisthorchis viverrini*, epidemiology, polyparasitism, food-borne trematode infections, *Taenia* spp., Lao PDR

**Introduction**

Food borne trematode infection is a worldwide health concern. The disease is endemic in many parts of the Far-East, South-East Asia and Eastern Europe and is transported over large distances in infected fish (Yossepowitch et al. 2004). The main public health problem is the infection of trematode belonging to the family *Opisthorchiidae* such as *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis*. An estimated 9 million people are infected with *O. viverrini*, 1.6 million of *O. felineus* and 35 million with *C. sinensis* (Lun et al. 2005; Sithithaworn & Haswell-Elkins 2003; WHO 1995). *C. sinensis* is endemic in China, Korea, Taiwan, Vietnam and Japan and *O. felineus* is widespread in Russian Federation and Eastern Europe. *O. viverrini* is of public health importance in South East Asia, in particular in Lao People’s Democratic Republic (Lao PDR) and Thailand (Sithithaworn & Haswell-Elkins 2003).

In Lao PDR, it is estimated that over 2 million people are infected with *O. viverrini* (WHO 1995). The parasite was first diagnosed in 1929 by Bedier and Chesneau in the cities Thakek (Khammouane province) and Vientiane capital where infection rates of 23% and 15% were found, respectively (Upatham & Viyanant 2003). Today, the prevalence is still very high. Rim and colleagues (Rim et al. 2003) conducted a nationwide survey in school children. It showed that the southern provinces have highest infection rates, exceeding 50% in school children. Follow-up survey in the city of Thakek showed that the prevalence rate increase to approximately 60% (Kobayashi et al. 2000).
Essential knowledge on *O. viverrini* infection, epidemiology and clinical implications has been gain over the last decade in neighbouring Thailand. There prevalence rates of *O. viverrini* vary between very low the South and 3.8% in the Central provinces. The highest prevalence are found towards the borders of Lao PDR in the North with 19.3% and in the North East with 15.7% infected individuals (Sithithaworn & Haswell-Elkins 2003; Upatham & Viyanant 2003). In latter area a large part of the population belong to the Lao-Thai ethnic group where raw food consumption is a common habit (Upatham & Viyanant 2003).

The adult *O. viverrini* parasite provokes hepatobiliar disease due to its localisation. Most infections are free of symptoms (Mairiang & Mairiang 2003). But clinical manifestations vary from non-severe such as right hypochondria pain, intestinal irritation to severe manifestation such as cholangitis, choletithiasis and cholecystitis. *Opistorchis* infection is a major risk factor for cholangiocarcinoma (Pinlaor et al. 2004; Sriamporn et al. 2004). Over 60% of this cancer is attributed to *O. viverrini* (Honjo et al. 2005).

*O. viverrini* eggs are shed with the faeces. A freshwater snails of the genus *Bithynia* (SADUN 1955) as a first and a *cyprinoid* fish species as second intermediate hosts (Haswell-Elkins et al. 1992) are required to fulfil its life cycle. The transmission of the parasite is granted when inadequate sanitation facilities and defecation habits are prevalent and the consumption of raw or insufficiently cooked fish is present.

The present study aimed to document the local epidemiology of opisthorchiasis in a rural district in Lao PDR in order to gather in-depth knowledge to develop detailed intervention strategies. A cross-sectional study was conducted in highly endemic district of Southern Lao PDR.
Materials and methods

Study area

The survey was conducted in the Saravane district, province of Saravane, located in Southern Lao PDR (Figure 1) where high infection rates in schoolchildren were reported (Rim, Chai, Min, Cho, Eom, Hong, Sohn, Yong, Deodato, Standgaard, Phommasack, Yun, & Hoang 2003). The district capital is also the capital of the province which has a total population of approximately 318,100 people. 20% of the total population are children under five years. The annual birth rate and mortality rate of this province is 39.8 and 8.3 per 1,000 people per annum, respectively. Only 61.9% have access to the safe drinking water and an estimation of 15.1% has any kind of sanitary facility at home (NSC 2003;PHO 2002). Saravane district is located in the low plain. An estimated population of 83,198 peoples (42,265 women, 40,933 men) live in 168 villages. 65.0% of population belong to Lao-Theung ethnic group (PHO 2002). This district has a main river (Xedone) with five important tributaries (Xeseth, Xekhone, huay-pao, Hauy-pa-ae and Hauy-sleng). All of them are extensively used for fishing. In the Xeseth River, the second largest dam of Lao PDR is present. The Xeset Hydropower Plant is a run-of-river scheme utilizing about 1.5 kilometers of the river with a drop of 157 meters. The plant provides electricity for the southern provinces of Lao PDR and Thailand and feeds the local irrigation systems. It is also extensively used for fishing purposes.

Study design and population surveyed

In February, March 2004 a cross-sectional study was conducted in the district. Households were selected in a two stage random sampling procedure. Firstly, 13 villages were randomly selected from the list of villages at the District Health Office, district of Saravane. Secondly, in each village 15 households were randomly selected from the list of households of the head of the
village. All members of the selected households older than 6 months and present on the survey day were enrolled in the study.

Field and laboratory investigation

Each study participant underwent a short clinical assessment by a general physician and was interviewed on socio-demographic features and risk factors for *O. viverrini* infection. The clinical assessment consisted of a physical examination in relation to hepatobiliar and intestinal symptoms and an interview about past and current illnesses. Age, level of education, profession, utilisation of sanitary household facilities and food (habit of eating raw or insufficiently cooked fish) and personal hygiene were information collected from each person. Availability of toilets or latrines and information on food preparing habits were obtained for each household by interviewing the head of the household. Parents or caretakers were interviewed for their children under 10 years of age.

A stool sample was obtained of each individual. A single Kato-Katz thick smear was established and examined on the presence of intestinal parasites (Katz *et al.* 1972). *O. viverrini* egg counts were obtained for each sample. The presence/absence of *Ascaris lumbricoides*, *Trichuris trichiura*, *Taenia* spp. and hookworms was recorded.

In each village, a fisher man was interviewed on the availability of fish and fish species in the village using a standardised questionnaire. A picture manual of local fish species of Lao PDR (LARReC 2000) was used to identify fish species based on reports of fishermen.

A sample of fishes per species and village was obtained and dissected into small scraps, pressed under a cover slide and examined for the presence of trematode metacerariae using a light microscope (Rhee *et al.* 1983).
Data management and statistical analysis

All data was entered in *EpiData 3.01* freeware (www.epidata.dk). Analysis was performed using STATA 8 (Stata Cooperation, College Station, TX, USA). The study participants were subdivided into seven age groups, namely (1) less than 6 years, (2) 6-15 years, (3) 16-25 years, (4) 26-35 years, (5) 36-45 years, (6) 46-55 years and (7) older than 55 years. Prevalence rates were used to report on helminth infections and risk factors. Standard statistical procedures were used to compare proportions and means. Geometric mean of egg counts with *O. viverrini* was calculated for infected persons. *O. viverrini*-positive individuals were grouped into three categories: light infections (1-999 eggs/gram of faeces [epg]), moderate infections (1000-9’999 epg) and heavy infections (>10,000 epg) according to a classification proposed by Maleevong and colleagues (Maleewong et al. 1992). Associations between *O. viverrini* infection and risk factors were performed on grouped data at village level and on individual data. Analyses on grouped data were correlations between prevalence of infection and prevalence of risk factors. Multivariate logistic regression was applied to relate *O. viverrini* infection and risk factors at individual level. 95% confidence intervals (CI) are provided where appropriate.

(NSC 2003)

Results

Study population

A total of 814 persons of 157 households and 13 villages were investigated. 65.2% (513) and 34.8 % (283) were ethnic Laoloum and Laotheung, respectively. 51.5% (419) were females (sex ratio M/F: 0.94) and 18.9% (159) were children below 6 years of age. Age ranged from 6 months to 98 years with a median age of 16 years. The average number of enrolled household members was 5.2 persons per household.
Among the 157 head of households 69.4% were male (Table 1). The median age was 43.0 years (45.0 years for males and 39.0 years for females, p = 0.01). Almost all head of households were married (86.6%) and their illiteracy rate was 31.2%. The illiteracy rate of women was two-fold higher than males (47.9% vs. 23.9%, p = 0.001). Subsistence farming was reported by most head of households (91.7%) whereas government employees (1.9%) and traders (1.9%) were rare.

Sanitation facilities

The availability of sanitation facilities in the household was very low. Overall, only 4.5% of the households had any toilette facility (Table 2). Only in one village, which was closest to the provincial capital, sanitation facilities were available. 95.0% (773 of 814) of all study participants reported to regularly defecate out-door. Of these, 92.1% defecated in the forest surround their village, 4.3% into a dugout hole, 3.1% any where and 0.5% into a river.

Clinical exam

In 23.6% (192/814) of the study participants a physical abnormality was diagnosed. 11.2% (91/814) and 3.8% (31/814) reported a diarrhoeal episode (more than 3 bowel movements within 24 hours) in the past one week and weight-loss, respectively. In 3.7% (30/814) a skin-eruption and in 2.7% (22/814) a chronic itching (chronic urticaria) was found. In 2.0% (16/814) of the enrolled persons a clinical anaemia (sub-conjunctiva pallor) and in 0.1% (2/814) a sub-icterus and hepatomegaly was diagnosed.

Faecal analysis

The prevalence rates of intestinal parasitic infections are given in Table 2. The most prevalent parasite infection was *O. viverrini*. In more than half of the study participants (58.5%) an infection was found. In all study villages opisthorchiasis infections were prevalent ranging from
14.3% to 79.9% per village. Prevalence rates were lowest in the two villages closest to the district capital: Benxeseth (14.3%) and Km2 (26.7%). O. viverrini infection increased with age (Figure 3). The infection rate did not differ between sexes (male 59.5% versus female 57.5%, p=0.570).

476 individuals tested positive for O. viverrini infection. 92.5% of whom were classified as having a light infection, 7.3% moderate infection and 0.2 % was classified as being heavily infected. The geometric mean eggs per gram stool was 154.3 (range 24-15,552 epg). There was no statistical different of mean eggs counts per gram stool between sexes (male 172.3 epg versus female: 138.6 epg, p = 0.357). The intensity of infection increased with age (Figure 3). The preschool children (aged < 6 years) had the lowest intensity of infection (90.7 epg), while the highest intensity was found in adults aged 45-55 years (206.6 epg).

Hookworm infection was found in 46.1% of subjects. Its highest prevalence at village level reached 79.6% (Table 2). A. lumbricoides and T. trichiura was detected in 16.0% and 11.0% of the samples, respectively. Eggs of the Taenia spp. were recorded in 5.0% of the exams. In three villages the prevalence of this parasite attained 10% of the study population.

In 83.5% (680/814) of the stool specimens analysed, at least one intestinal parasite species was found. 40.9% (333/814) of the persons were infected with one, 33.1% (269/814) with two, 8.9% (73/814) with three and 0.6% (5/814) with four different parasite species. The infection rates did not differ between sexes (83.5% vs. 83.5%, p = 0.996).

*Fish consumption habits*

75.2% (611/814) of persons reported to frequently consume raw or insufficiently uncooked fish (Table 2). The habit was observed in all villages with rates between 44.6 and 91.2%. Men reported this habit with a slightly higher frequency (79.2% vs. 71.3%, p = 0.009). The reporting
rate of this nutritional habit showed a marked increase with age parallel to the infection rates (Figure 2).

*Fish examination*

43 different fish species were found in the study villages. Of these, 23 species (53.4%) belonged to the family of *cyprinoidiae*. A sample of 98 fishes of *cyprinoidiae* fish originating of 6 different rivers was dissected for the presence of metacercariae. 59.2% (58/98) of inspected fish were found infected. Only fish of three species were not found infected (Lao name in bracket): *Cyprinus carpiolinnaeus (Pa-nai)*, *Oreochromis niloticus (Pa-nin)* and *Barbichthys nitibus (Pa-soy)*

In seven species high metacercaria infection rates were found 39/49 (79.6%). The following fish species belonged to this group (Lao name in bracket): *Hampala macrolepidota (Pa-sout)* 7/7 (100.0%), *Lobocheilus melanotaen (Pa-langnam)* 9/9 (100.0%), *Poropuntius cf laoensis (Pa-chat)* 9/10 (90.0%), *Puntius brevis (Pa-khaomon)* 6/7 (85.7%), *Cyclocheilichthys enoplos (Pa-chok)* 4/5 (80.0%), *Oreichthys parvus smit (Pa-siew-na)* 2/5 (40.0%) and *Rasbora Ourotacniatiran (Pa-siew-our)* 2/6 (33.3%).

Infection were rarely detected 19/49 (36.8%) in the following fish species (Lao name in brackets): *Amblynchichthys trunca (Pa-tapo)*, *Amblynchithys truncatus (Pa-mang)*, *Cyclocheiliehtysarmatus (Pa-dokgneue)*, *Dicerodontusahme (Pa-hangdeng)*, *Garra fuligirosa fowler (P-khome)*, *Hypsibarbus pierrei (Pa-park)*, *Notopterus (Pa-tong)*, *Osteochilus schlegeli (Pa-namom)*, *Osteochilus hasselti (Pa-ithai)*, *Parachela maculicuda (Pa-tebhuabird)*, *Puntioptites falcifer (Pa-sakang)*, *Raiamas guytatua (Pa-mitsanak-noy)*, *Systomus orphoides (Pa-pok)*.
Associations with *O. viverrini* infection

Analysis on grouped data on village level showed a strong positive correlation between the prevalence of *O. viverrini* infection and the habit of consuming raw fish or insufficiently cooked fish \((r=0.76, \ p=0.003, \text{Figure 3})\). No correlation was found with the rate of persons who reported not having heard about opisthorchiasis \((r=0.18, \ p=0.564)\). Moderate negative correlations were found with prevalence of hookworms \((r=-0.57, \ p=0.041)\), *T. trichiura* \((r=-0.69, \ p=0.009)\) and *A. lumbricoides*. Latter was not significant \((r=-0.57, \ p=0.060)\). No correlation was found with *Taenia spp.* infection rates \((r=0.03, \ p=0.918)\).

In all age groups the consumption of raw or insufficiently cooked fish was reported in rates above 75%, except in children under 6 years of age (Figure 2).

Logistic regression was used to investigate association of risk factors and *O. viverrini infection* at the individual level (Table 4). The following predictors were included in the regression model: age, sex, habit of eating raw or insufficiently cooked fish, availability of latrine at home and educational level of head of households. The analysis revealed that age, fish consummation habits and availability of latrines were independently contributing to the risk of infection with *O. viverrini*.

Schoolchildren aged 6 to 15 years had 2.52 times \((p=0.001)\) greater risk for an infection with *O. viverrini* than preschool children (aged < 6 years). Likewise adults aged 46-55 years had a 12.57 fold increased risk \((p<0.001)\). Study participants who reported to consume raw or insufficiently cooked fish had 2.31 times \((p<0.001)\) higher risk of *O. viverrini* infection. Having a sanitation facility in the household was associated with a 74% risk decrease \((OR: \ 0.26, \ p=0.001)\).

Sex did not contribute to the overall risk of infection \((OR: \ 1.18, \ p=0.318)\). Study participants with a maximum level of education of primary school had a 79% increased risk for an *O.
*O. viverrini* infection (OR: 1.79, p=0.004) while in people with secondary school level education or higher and reveal increased risk was seen (p=0.955).

Two cases of sub-icterus and hepatomegalia findings were diagnosed in the clinical examination. Both were infected with *O. viverrini*, one person with a light and one with moderate intensity of infection.

**Discussion**

*Prevalence and impact of O. viverrini*

Stool examinations show extremely high infection rates of *O. viverrini*. This finding confirms the previous reports by Rim and colleagues in the province of Saravane (Rim, Chai, Min, Cho, Eom, Hong, Sohn, Yong, Deodato, Standgaard, Phommasack, Yun, & Hoang 2003) and also Kobayashi and colleagues who showed high infection rates in the province of Khammouane (Kobayashi, Vannachone, Sato, Manivong, Nambanya, & Inthakone 2000). In addition our study is most likely an underestimating of the infection rates. Only one stool sample per person could be examined. Repeated stool analysis with Kato Katz smear technique has shown to increase substantially the sensitivity (de Vlas & Gryseels 1992; Marti & Koella 1993), hence we can assume that the “true” prevalence is considerably higher.

However, misdiagnosis of *O. viverrini* by egg detection can occur. Small intestinal trematodes such as species of the genera of *Haplorchis* have morphologically very similar eggs. Using Kato-Katz technique the difference can not be made easily and confusion can not be excluded. *Haplorchis taichui and H. yokogawai* are endemic in Lao PDR. However they were found in much lower prevalence than *O. viverrini* (Ditrich et al. 1990; Giboda et al. 1991a; Giboda et al. 1991b). The clear extend, however, of their importance is not known and needs further clarifications.
*O. viverrini* infection is a major risk factor for cholangiocarcinom (Honjo, Srivatanakul, Sriplung, Kikukawa, Hanai, Uchida, Todoroki, Jedpiyawongse, Kittiwatanachot, Sripa, Deerasamee, & Miwa 2005). The prevalence rate of Saravane district is considerably higher than recent estimates from districts of North-eastern Thailand (Sriamporn, Pisani, Pipitgool, Suwanrungruang, Kamsa-ard, & Parkin 2004). In these districts the incidence of cholangiocarcinoma reached 302 cases per 100,000 populations (Sriamporn, Pisani, Pipitgool, Suwanrungruang, Kamsa-ard, & Parkin 2004). In addition recent, finding suggest that 60 % of the cholangiocarcinom can be attributed to *Opisthorchis* infection (Honjo, Srivatanakul, Sriplung, Kikukawa, Hanai, Uchida, Todoroki, Jedpiyawongse, Kittiwatanachot, Sripa, Deerasamee, & Miwa 2005). Based on these and demographic data of the district of Saravane up to 152 cases of cholangiocarcinom per year due to *Opisthorchis* infection must be expected for the district of Saravane alone.

Although *O. viverrini* was observed in high prevalence rates the intensity of infections was low. An observation which was made consistently also in Thailand (Sithithaworn and Haswell-Elkins 2003). This does not necessarily mean that the public health impact is low. The evaluation of the risk for cholangiocarcinoma performed by Honjo and colleagues (Honjo, Srivatanakul, Sriplung, Kikukawa, Hanai, Uchida, Todoroki, Jedpiyawongse, Kittiwatanachot, Sripa, Deerasamee, & Miwa 2005) was based on the presence of serological antibody and therefore even light infections and transient infections may lead to liver cancer. In our study only few persons were detected with symptoms associated with other hepatobiliar disease such as jaundice. This corresponds to earlier findings from Thailand (Pungpak *et al.* 1989). However, recent community based ultrasonographic studies showed that hepatobiliar abnormalities are associated with the *O. viverrini* infection and are possible precursor conditions for cholangiocarcinom (Elkins *et al.* 1996).
Our study draws attention to the sharp increase in infection rates, intensity of infection and the habit of eating raw or insufficiently cooked fish by age and showed a slight decrease in the oldest age groups. This finding suggests an accumulation of infection over time due to continues exposure. Furthermore, no notable differences are seen between sexes. Comparable observations were made in the neighboring Thai setting as a recent review of Sithithaworn and Haswell-Elkins showed (Sithithaworn & Haswell-Elkins 2003).

Furthermore, our study demonstrates a high rate of multi-helminth infections of the intestine. These infections in their turn contribute to additional functional and developmental morbidity (Raso et al. 2005).

Human infection occurs through ingestion of infectious meta-cercariae of fish. Our investigation draws attention to the fact that a large number of different cyprinoid fish species are consumed regularly by the rural population and that most of them may harbour infectious stages. This information is in concordance with the WHO report (WHO 1995) in which more then 80 cyprinoid fish species are listed as potential second intermediate fish species. Cyprinoid fish of genera Puntius, Cyclocheilichtys and Hampala reportedly to be highly infected with O. viverrini meta-cercaria (WYKOFF et al. 1965) have also shown high infection rates in our study. Unfortunately, we were not able to employ the pepsin digestion technique for the meta-cercarial diagnosis (Tesana et al. 1985) and hence were not able to determine the exact parasite species. Some of the infection may therefore be also be due to other trematode infections.

Cats, dogs and rats are also definitive hosts for O. viverrini (SADUN 1955). The relative contribution of these hosts to the transmission of the parasite to the intermediate snail hosts are not known but are considered as minor compared with the higher egg excretion and hygiene behaviour of humans (SADUN 1955). Estimations of infection rates in these animals have not been studied and remain unknown for Lao PDR.
Risk factors for *O. viverrini* infection

The preference for raw and insufficiently cooked fish dishes, the low availability of sanitation facilities and the low instruction level are main factors contributing to a high prevalence level of *O. viverrini*.

Furthermore, the overall illiteracy rate of head of households was very high. This might be an underlying key factor for parasitic infections. Many surveys confirm that educational level of the parents is significantly associated with the intestinal parasitic infection. The current survey found the lower prevalence for an *O. viverrini* infection in the households with the high educational level of head of households but this finding was not statistically. Results of a survey conducted in Iran showed that with increased educational level of parents, the infection rate declined more than 200% (Nematian *et al.* 2004). Other studies have noted that the infection of *A. lumbricoides* was significantly less prevalent in the educated parents (Holland *et al.* 1996; Tshikuka *et al.* 1995).

In general, lack of sanitation is a key determinant for helminth infections. Its correlation is well documented (Esrey *et al.* 1991; Fewtrell *et al.* 2005). Our current survey shows that almost all of the population included toilette facilities were lacking and virtually all study participants reported the habit of defecating out-door.

Eating raw or insufficiently cooked fish is widespread in Lao culture. Our survey confirms the affinity of the population surveyed and demonstrates an extremely high rate with which this habit is prevalent in this rural district even today. This is the route of infection for opisthorchiasis. The habit is eating raw or undercooked fish is prevalent in other countries of South-East Asia (Anantaphruti 2001).
O. viverrini is highly endemic in the district of Saravane. This finding demonstrate the risk factors such as the consumption of raw of insufficiently cooked fish, the absence of sanitation facilities and knowledge on O. viverrini are highly prevalent in the district. Interventions strategies focussing on mass-treatment, sanitation and health education on habits to consume raw fish have shown in Thailand to be effective (Jongsuksuntigul & Insomboon 2003). They are urgently needed in Saravane district and similarly endemic areas of Lao PDR.

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References


LARReC. Manual of local fish in different setting in Lao PDR. 2000.


PHO. Health Statistic of Province of Saravane, Provincial Health Office. 2002.


Table 1: Characteristics of studied households (n=157)

<table>
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<tr>
<th></th>
<th>Total (n = 157)</th>
<th>Male (n = 109)</th>
<th>Female (n = 48)</th>
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<td><strong>Age in years</strong></td>
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<td>Median</td>
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<td>45.0</td>
<td>39.0</td>
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<td>25.0-81.0</td>
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<td><strong>Ethnic group % (95% CI)</strong></td>
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<td>Laoloum</td>
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<td>50.0 (35.2-64.8)</td>
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<td>Laotheung</td>
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<td>33.9 (25.1-43.6)</td>
<td>50.0 (35.2-64.8)</td>
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<td><strong>Education % (95% CI)</strong></td>
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<td>61.5 (51.7-70.6)</td>
<td>43.8 (29.5-58.8)</td>
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<td>1.3 (0.2-4.5)</td>
<td>0.9 (&lt; 0.1-5.0)</td>
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<td>Farmer</td>
<td>91.7 (86.3-95.5)</td>
<td>90.8 (83.8-95.5)</td>
<td>93.8 (82.8-98.7)</td>
</tr>
<tr>
<td>Trader</td>
<td>1.9 (0.4-5.5)</td>
<td>2.8 (0.67.8)</td>
<td>0</td>
</tr>
<tr>
<td>Government employee</td>
<td>1.9 (0.4-5.5)</td>
<td>2.8 (0.67.8)</td>
<td>0</td>
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</tbody>
</table>
Table 2: Prevalence of intestinal parasites and selected risk factors by village (n=814)

<table>
<thead>
<tr>
<th>Villages</th>
<th>O. viverrini</th>
<th>Hookworm</th>
<th>A. lumbricoides</th>
<th>T. trichiura</th>
<th>Taenia spp.</th>
<th>Habit of eating raw or insufficiently cooked fish</th>
<th>Never heard about O. viverrini</th>
<th>Availability of toilette at home</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kengsim</td>
<td>79.7</td>
<td>29.0</td>
<td>1.5</td>
<td>4.4</td>
<td>5.8</td>
<td>79.7</td>
<td>88.4</td>
<td>0.0</td>
</tr>
<tr>
<td>NongMakyYang</td>
<td>78.3</td>
<td>43.5</td>
<td>1.5</td>
<td>5.8</td>
<td>2.9</td>
<td>87.0</td>
<td>88.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Napari</td>
<td>70.7</td>
<td>40.0</td>
<td>52.0</td>
<td>1.3</td>
<td>1.3</td>
<td>69.3</td>
<td>98.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Nabone</td>
<td>68.8</td>
<td>29.9</td>
<td>2.6</td>
<td>9.1</td>
<td>10.4</td>
<td>87.0</td>
<td>83.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Channeua</td>
<td>66.7</td>
<td>54.0</td>
<td>0.0</td>
<td>14.3</td>
<td>4.8</td>
<td>74.2</td>
<td>96.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Thamuongkao</td>
<td>62.3</td>
<td>39.1</td>
<td>0.0</td>
<td>2.9</td>
<td>10.1</td>
<td>73.9</td>
<td>66.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Khoksavath</td>
<td>59.7</td>
<td>47.4</td>
<td>1.8</td>
<td>1.8</td>
<td>0.0</td>
<td>91.2</td>
<td>71.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Nava</td>
<td>59.6</td>
<td>32.7</td>
<td>9.6</td>
<td>1.9</td>
<td>3.9</td>
<td>86.5</td>
<td>62.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Natum</td>
<td>59.5</td>
<td>52.7</td>
<td>8.1</td>
<td>4.1</td>
<td>1.4</td>
<td>86.5</td>
<td>51.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Leunbok</td>
<td>44.9</td>
<td>79.6</td>
<td>59.2</td>
<td>32.7</td>
<td>8.2</td>
<td>73.5</td>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Khokkao</td>
<td>42.4</td>
<td>55.9</td>
<td>0.0</td>
<td>1.7</td>
<td>10.2</td>
<td>50.9</td>
<td>88.1</td>
<td>0.0</td>
</tr>
<tr>
<td>km2</td>
<td>26.7</td>
<td>40.0</td>
<td>20.0</td>
<td>11.1</td>
<td>4.4</td>
<td>62.2</td>
<td>48.8</td>
<td>70.0</td>
</tr>
<tr>
<td>Benxeseth</td>
<td>14.3</td>
<td>67.9</td>
<td>62.5</td>
<td>66.1</td>
<td>1.8</td>
<td>44.6</td>
<td>91.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>58.5</td>
<td>46.1</td>
<td>15.7</td>
<td>11.1</td>
<td>5.0</td>
<td>75.2</td>
<td>80.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>
### Table 3: Results of multivariate analysis for risk factors of *O. viverrini* infection

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group (versus below 6 years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-15 years</td>
<td>2.52</td>
<td>0.001</td>
<td>1.45-4.40</td>
</tr>
<tr>
<td>16-25 years</td>
<td>5.60</td>
<td>&lt;0.001</td>
<td>2.88-10.92</td>
</tr>
<tr>
<td>26-35 years</td>
<td>5.84</td>
<td>&lt;0.001</td>
<td>3.00-11.48</td>
</tr>
<tr>
<td>36-45 years</td>
<td>8.07</td>
<td>&lt;0.001</td>
<td>3.96-16.46</td>
</tr>
<tr>
<td>46-55 years</td>
<td>12.57</td>
<td>&lt;0.001</td>
<td>5.18-30.50</td>
</tr>
<tr>
<td>&gt; 55 years</td>
<td>9.40</td>
<td>&lt;0.001</td>
<td>4.52-19.56</td>
</tr>
<tr>
<td><strong>Gender (versus female)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.18</td>
<td>0.318</td>
<td>0.85-1.64</td>
</tr>
<tr>
<td><strong>Habit of eating raw fish (versus “no”)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.31</td>
<td>&lt;0.001</td>
<td>1.57-3.40</td>
</tr>
<tr>
<td><strong>Presence of any sanitation facility at home (versus “absent”)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitation facility present</td>
<td>0.26</td>
<td>0.001</td>
<td>0.11-0.57</td>
</tr>
<tr>
<td><strong>Educational level (versus “no schooling”)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>1.79</td>
<td>0.004</td>
<td>1.20-2.66</td>
</tr>
<tr>
<td>Secondary school &amp; higher</td>
<td>1.02</td>
<td>0.955</td>
<td>0.54-1.91</td>
</tr>
</tbody>
</table>
Figure 1: Map of Lao PDR and Saravane district
Figure 2: Association between the prevalence of *O. viverrini* infection and the consumption of raw or insufficiently cooked fish at village level (n = 13)
Figure 3: Prevalence (%) and intensity (eggs per gram stool) of infection with *O. viverrini* and prevalence (%) of eating raw or insufficiently cooked fish by age.