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# Diversity of human intestinal helminthiasis in Lao PDR

**Somphou Sayasone[a,b], Youthanavane Vonghajack[c], Monely Vanmany[b], Oroth Rasphone[d], Smarn Tesana[e], Jürg Utzinger[a], Kongsap Akkhavong[b], Peter Odermatt[a,*]**

[a] *Department of Public Health and Epidemiology, Swiss Tropical Institute, P.O. Box, 4002 Basel, Switzerland*
[b] *National Institute of Public Health, Ministry of Health, Vientiane, Lao PDR*
[c] *Department of Parasitology, Faculty of Medicine, University of Health Sciences, Vientiane, Lao PDR*
[d] *Department of Radiology, Mahosot Hospital, Ministry of Health, Vientiane, Lao PDR*
[e] *Food-Borne Parasite Research Group, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

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Polyparasitism;
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**Summary** Food-borne trematodiasis is an emerging public health problem, including in Lao PDR. We investigated the diversity of intestinal helminthes and polyparasitism in patients with hepatobiliary or intestinal symptoms in hospital and community-based surveys. Stool samples from 232 individuals aged ≥15 years were examined by the Kato-Katz method (three samples) and a formalin ethyl-acetate concentration technique (one sample). *Opisthorchis viverrini* and minute intestinal flukes (MIF) were common, with prevalences of 86.2% and 62.9%, respectively. Hookworm was the predominant soil-transmitted helminth (65.9%). The prevalences of *Taenia* spp., *Strongyloides stercoralis* and *Trichuris trichiura* were 22.8%, 10.3% and 8.6%, respectively. Additionally, 97 individuals were purged; *O. viverrini* and *Haplorchis taichui* were found in 95 and 76 participants, respectively. Other trematodes included *Phaneropsolus bonnei* (22.7%), *Prosthodendrium molenkampi* (14.4%), *Haplorchis pumilio* (5.2%), *Haplorchis yokogawai* (3.1%) and *Echinochasmus japonicus* (3.1%). Co-infection with *O. viverrini* and MIFs was rampant (81.4%). Polytrematode infection is highly prevalent in Lao PDR and hence requires urgent attention.


## 1. Introduction

Food-borne trematodes parasitising the liver, lungs and intestinal tract of humans are an emerging public health problem.[1–3] It is assumed that over one-half of infections

* Corresponding author. Tel.: +41 61 284 8214; fax: +41 61 284 8105.
*E-mail address:* peter.odermatt@unibas.ch (P. Odermatt).

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worldwide occur in Southeast Asia.[2] Two species of liver flukes, namely *Clonorchis sinensis* and *Opisthorchis viverrini*, and more than 50 species of intestinal flukes (species belonging to the genera *Echinostoma*, *Fasciolopsis* and *Haplorchis*) have been described in the literature.[4] An estimated 750 million individuals are at risk and approximately 40 million are infected, yet food-borne trematodiasis is often neglected.[2,5,6]

Lao PDR is situated in central Southeast Asia, with the Mekong River running through the country from north to south. The Mekong River basin with its widely interconnected ecosystems has been identified as a high-risk area for food-borne trematodiasis. Previous studies have shown that *O. viverrini* is endemic in all provinces of Lao PDR.[7] The highest prevalence rates were found in central and southern parts, reaching levels of up to 60%.[8] However, in these studies only '*Opisthorchis*-like' eggs were reported. Minute intestinal flukes (MIF) have eggs that are similar to *O. viverrini* in terms of morphology and size and hence species-specific diagnosis is difficult.

Adult *O. viverrini* live in the bile duct and can cause chronic inflammation. As a result, cellular injury and partial obstruction of bile flow may occur.[9] However, the majority of individuals with an *O. viverrini* infection are asymptomatic. In approximately 5—10% of infected individuals, usually among those with heavy infection intensities, non-specific and non-severe clinical manifestations occur, e.g. right hypochondrial pain and intestinal irritation. The most severe manifestations include cholangitis, cholecystitis and cholelithiasis.[8—10] Moreover, *O. viverrini* is an established risk factor for cholangiocarcinoma; indeed, the highest incidence of cholangiocarcinoma in the world has been found in northeast Thailand, with >60% being attributed to *O. viverrini*.[9,11,12]

In addition to *O. viverrini*, other food-borne trematodes with intestinal tropism occur in Lao PDR, the most prominent of which is *Haplorchis* spp.[13,14] Stool analysis of Lao patients treated for 'opisthorchiasis' in Czechoslovakia, as well as a recent survey conducted in the central and southern parts of Lao PDR, revealed that a significant number of patients suffered from opisthorchiasis and haplorchiasis concurrently.[13,14] The co-occurrence of *O. viverrini* and *Haplorchis* spp. is not only a challenge for diagnosis but may also aggravate morbidity.

In the present study, we assessed the diversity of trematode infections and intestinal polyparasitism during hospital- and community-based cross-sectional surveys carried out in Lao PDR. Additionally, a subsample of patients was purged for adult worm collection and species identification. Hence, our study allowed validation of the quantitative formalin ethyl-acetate concentration technique (FECT) for diagnosing *O. viverrini* and MIFs.

## 2. Materials and methods

### 2.1. Study area and population

A hospital-based study was carried out at the infectious disease wards of Mahosot Hospital (Vientiane, Lao PDR) and Savannakhet Provincial Hospital (Savannakhet, Lao PDR) in September and October 2005. Mahosot Hospital is the national university leading hospital, with 450 beds. Savannakhet Provincial Hospital, with 80 beds, is the largest provincial hospital in Lao PDR and serves as a referral hospital for the southern and central provinces. All patients aged ≥15 years who were hospitalised with hepatobiliary or intestinal symptoms such as icterus, stomach ache, abdominal pain (right hypochondrial quadrant), nausea, vomiting and abdominal irritation were invited to participate.

Community surveys were carried out in Khamsida (Champhone district, Savannakhet province) and Thamouangkao (Saravane district, Saravane province) in November 2005 and June 2006, respectively. *Opisthorchis viverrini* is highly endemic in both areas.[7,8] Khamsida is located ~15 km from the central district (population 584) and Thamouangkao ~10 km from Saravane (estimated population 1000). Residents in both communities are primarily engaged in farming. Main dishes are prepared with raw or insufficiently cooked fish and other aquatic products.[8] In these community-based surveys, the same inclusion criteria were applied as in the hospital-based surveys.

Written informed consent was obtained from each participant prior to enrolment.

### 2.2. Field and laboratory procedures

Demographic (e.g. age, sex, educational attainment and professional activity) and behavioural data (e.g. food consumption habits) were obtained with a pre-tested questionnaire. All study participants underwent a physical examination by a general physician.

Three stool specimens, collected over consecutive days, were obtained from each individual. One Kato-Katz thick smear (41.7 mg) was prepared from each specimen.[15] Slides were allowed to clear for 30 min prior to examination under a light microscope. The number of eggs was counted and recorded for each parasite species separately.

Exactly 300 mg of stool was placed in a small tube containing 10 ml of sodium acetate—acetic acid—formalin.[16] Samples were subjected to quantitative FECT at the parasitological department of the Faculty of Medicine, University of Health Sciences (Vientiane, Lao PDR), assisted by laboratory staff from the Swiss Tropical Institute (Basel, Switzerland). Helminth eggs were counted and recorded for each species separately. *O. viverrini* eggs were differentiated from those of MIFs by demonstrating the distinct shoulders at the operculum as well as by eggshell and knob morphology under a light microscope at high magnification.[17]

### 2.3. Purgation of patients

A subsample of 97 individuals with heavy '*Opisthorchis*-like' infections was enrolled in a purging procedure.

Each individual received a single 40 mg/kg oral dose of praziquantel after dinner. Albendazole 400 mg was added if patients were co-infected with soil-transmitted helminths. All stools produced after treatment were collected. The following morning, 45 ml of monosodium sulphate solution was administered to patients (Swiff; Berlin Pharmaceutical Industry Co. Ltd., Berlin, Germany). All successive diarrhoeal stools within 24 h (usually six to eight bowel

movements) were collected and examined. Bottled drinking water was provided and patients were encouraged to drink as much as possible.

### 2.4. Worm collection

Diarrhoeal stool was poured into a 2 l bottle, filled up with tap water and stirred until the stool was homogenously mixed. After sedimentation for 10 min, the supernatant was discharged, water was added and stirred again. This washing procedure was repeated until the supernatant became clear. The sediment was examined for the presence of adult worms as follows. First, adult *Taenia* spp., *Echinostoma* spp. and *O. viverrini* worms were visually searched for. Second, the remaining sediment was examined with a stereomicroscope for the presence of MIFs. The number of species-specific parasites was recorded for each individual. Species identification was confirmed under a light microscope after specimens were coloured with carmine and mounted in Permount.

All individuals infected with *O. viverrini* and soil-transmitted helminth infections were treated according to national guidelines.[18] An antispasmodic treatment and oral rehydration was provided in case of side effects due to drug administration.

### 2.5. Data management and statistical analysis

Data were double-entered and validated in EpiData version 3.1 (EpiData Association; Odense, Denmark). Statistical analyses were performed with STATA version 9 (Stata Corp.; College Station, TX, USA). Those individuals with complete data records were included in the final analyses.

Age was subdivided into five groups: (i) 15—25 years; (ii) 26—35 years; (iii) 36—45 years; (iv) 46—55 years; and (v) >55 years. Infections with hookworm, *Ascaris lumbricoides*, *Trichuris trichiura* and *O. viverrini* were grouped into light, moderate and heavy infections, respectively, according to Maleewong et al.[19] and WHO guidelines[20]: hookworm, 1—1999, 2000—3999 and ≥4000 eggs per gram of faeces (epg); *A. lumbricoides*, 1—4999, 5000—49 999 and ≥50 000 epg; *T. trichiura*, 1—999, 1000—9999 and ≥10 000 epg; and '*O. viverrini*-like', 1—999, 1000—9999 and ≥10 000 epg.

Fisher's exact test and $\chi^2$ test were employed to investigate associations between categorical variables. ANOVA was used to associate the parasite egg counts with either age group or sex. The arithmetic mean of worm counts was calculated for infected individuals in purged patients. Linear regression and Spearman's correlation were used to investigate the relationship between number of adult worms of *O. viverrini* and MIFs and their egg counts in microscopic examination of stool samples. For all analyses the significance level was $P = 0.05$.

## 3. Results

### 3.1. Stool analysis

Complete parasitological data were obtained for 232 individuals, giving an overall compliance of 97.1%. The majority of subjects participated in the community survey ($n = 213$; 91.8%).

Table 1 summarises the results from the microscopic stool examination using either the Kato-Katz technique or FECT. Also shown are the pooled results, with all subsequent analyses performed on these pooled data. '*Opisthorchis*-like' eggs were diagnosed in 217 individuals (93.5%). Examination of multiple Kato-Katz thick smears resulted in a significantly higher helminth infection prevalence compared with a single stool specimen examined by FECT: hookworm (62.9% vs. 37.5%; $P < 0.001$); *T. trichiura* (7.8% vs. 1.7%; $P = 0.002$); and *Taenia* spp. (21.1% vs. 9.9%; $P = 0.001$). With regard to diagnosing '*Opisthorchis*-like' eggs, the two methods

**Table 1** Number (%) of individuals diagnosed with intestinal parasites using the Kato-Katz technique (three samples) and a formalin ethyl-acetate concentration technique (FECT) (one sample), and pooled results using both diagnostic approaches ($n = 232$).

| Parasite | Pooled results | Kato-Katz | FECT | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|
| Trematodes | | | | | |
| *Opisthorchis*-like eggs | 217 (93.5) | 216 (93.1) | 204 (87.9) | 3.61 | 0.057 |
| *Opisthorchis viverrini* | 200 (86.2) | ND | 200 (86.2) | NA | NA |
| Minute intestinal flukes | 146 (62.9) | ND | 146 (62.9) | NA | NA |
| Echinostomatidae | 51 (22.0) | 40 (17.2) | 38 (16.4) | 0.06 | 0.804 |
| Nematodes | | | | | |
| Hookworm | 153 (65.9) | 146 (62.9) | 87 (37.5) | 30.00 | <0.001 |
| *Strongyloides stercoralis* | 24 (10.3) | ND | 24 (10.3) | NA | NA |
| *Trichuris trichiura* | 20 (8.6) | 18 (7.8) | 4 (1.7) | 9.35 | 0.002 |
| *Enterobius vermicularis* | 2 (0.9) | 0 | 2 (0.9) | 2.01 | 0.156 |
| *Ascaris lumbricoides* | 1 (0.4) | 1 (0.4) | 1 (0.4) | 0.001 | 1.000 |
| *Capillaria philippinensis* | 1 (0.4) | 1 (0.4) | 1 (0.4) | 0.001 | 1.000 |
| Cestodes | | | | | |
| *Taenia* spp. | 53 (22.8) | 49 (21.1) | 23 (9.9) | 11.11 | 0.001 |
| *Hymenolepis diminuta* | 1 (0.4) | 1 (0.4) | 0 | 1.00 | 0.317 |

NA: not applicable; ND: not determined.

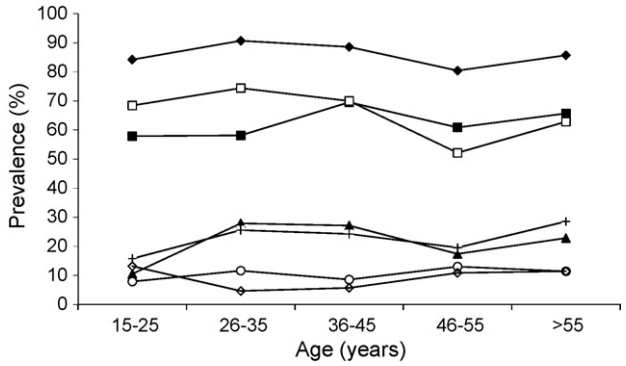


**Figure 1** Age—prevalence curves for *Opisthorchis viverrini* (♦), minute intestinal flukes (■), hookworm (□), Echinostomatidae (▲), *Taenia* spp. (+), *Strongyloides stercoralis* (◯) and *Trichuris trichiura* (◊) diagnosed by Kato-Katz and formalin ethyl-acetate concentration technique in 232 individuals.

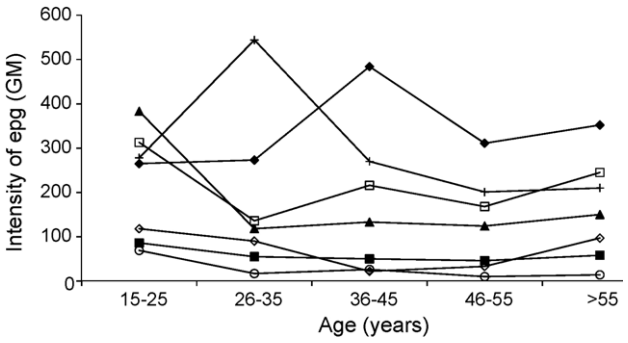


**Figure 2** Age—intensity [geometric mean (GM) of eggs per gram of faeces (epg)] curves for *Opisthorchis viverrini* (♦), minute intestinal flukes (■), hookworm (□), Echinostomatidae (▲), *Taenia* spp. (+), *Strongyloides stercoralis* (◯) and *Trichuris trichiura* (◊), diagnosed by Kato-Katz method and formalin ethyl-acetate concentration technique (FECT) in 232 individuals. *Opisthorchis viverrini*, minute intestinal flukes and *S. stercoralis* were only counted by FECT.

showed borderline statistical significance (93.5% vs. 87.9%; $P = 0.057$).

There was a tendency for male study participants to have a higher infection prevalence for individual parasites. Statistically significant sex differences were found for *O. viverrini* (males 91.6%, females 81.7%; $P = 0.030$), Echinostomatidae (males 29.9%, females 15.1%; $P = 0.006$) and *Strongyloides stercoralis* (males 18.7%, females 3.2%; $P < 0.001$).

Figure 1 depicts age-specific prevalence curves for the different parasites investigated. None of the helminth prevalences were significantly associated with age. A distinct age peak of intensity of infection was found for *O. viverrini* and *Taenia* spp. in the 36—45 years and 26—35 years age groups, respectively (Figure 2). None of the other helminths showed a statistically significant association between intensity of infection and age or sex.

## 3.2. Polyparasitism and infection intensity

Among the 232 individuals with complete data sets, only 15 (6.5%) were free of intestinal parasites. Single-species parasitic infections were found in 23 individuals (9.9%). Over two-third of the participants (67.2%) harboured two to four different parasite species concurrently. Thirty individuals (12.9%) were infected with five different parasites. Six individuals (2.6%) were infected with six different parasites and two individuals (0.9%) (males aged 15 years and 28 years) harboured seven parasite species.

Table 2 summarises infection intensities, expressed in epg, of the different intestinal parasites either detected by the Kato-Katz or FECT, or after combining the results from both diagnostic approaches. The pooled results showed that 117 (50.4%) of the examined patients showed an infection with ''*Opisthorchis*-like'' eggs of moderate intensity, whereas 20 individuals (8.6%) had a heavy infection.

## 3.3. Characteristics of purged individuals

From the 107 individuals invited for the purging study, 10 were excluded upon clinical examination as they were

**Table 2** Number (%) of individuals with no, light, moderate or heavy infection intensities with *Opisthorchis viverrini*, hookworm and *Trichuris trichiura* using the Kato-Katz technique (three samples) and a formalin ethyl-acetate concentration technique (FECT) (one sample), and pooled results ($n = 232$).

| Parasite | No infection | Light | Moderate | Heavy |
|---|---|---|---|---|
| Kato-Katz | | | | |
| *Opisthorchis*-like eggs | 16 (6.9) | 66 (28.4) | 112 (48.3) | 38 (16.4) |
| Hookworm | 86 (37.1) | 133 (57.3) | 11 (4.7) | 2 (0.9) |
| *Trichuris trichiura* | 214 (92.2) | 18 (7.8) | 0 | 0 |
| FECT | | | | |
| *Opisthorchis viverrini* | 32 (13.8) | 66 (28.4) | 85 (36.6) | 49 (21.1) |
| Hookworm | 145 (62.5) | 76 (32.8) | 8 (3.4) | 3 (1.3) |
| *Trichuris trichiura* | 228 (98.3) | 4 (1.7) | 0 | 0 |
| Pooled results | | | | |
| *Opisthorchis*-like eggs | 15 (6.5) | 80 (34.5) | 117 (50.4) | 20 (8.6) |
| Hookworm | 79 (34.1) | 145 (62.5) | 7 (3.0) | 1 (0.4) |
| *Trichuris trichiura* | 212 (91.4) | 20 (8.6) | 0 | 0 |

**Table 3** Number (%) of individuals with adult helminths recovered after purgation among 97 study participants in a community and hospital-based survey in Lao PDR.

| Parasite | Overall ($n = 97$) | Community ($n = 82$) | Hospital ($n = 15$) | $\chi^2$ | $P$-value |
|---|---|---|---|---|---|
| Liver flukes | | | | | |
| *Opisthorchis viverrini* | 95 (97.9) | 82 (100) | 13 (86.7) | 11.03 | 0.001 |
| Minute intestinal flukes | | | | | |
| *Haplorchis taichui* | 76 (78.4) | 69 (84.1) | 7 (46.7) | 10.29 | 0.001 |
| *Phaneropsolus bonnei* | 22 (22.7) | 20 (24.4) | 2 (13.3) | 0.92 | 0.336 |
| *Prosthodendrium molenkampi* | 14 (14.4) | 13 (15.9) | 1 (6.7) | 0.89 | 0.344 |
| *Haplorchis pumilio* | 5 (5.2) | 5 (6.1) | 0 | NA | NA |
| *Echinochasmus japonicus* | 3 (3.1) | 3 (3.7) | 0 | NA | NA |
| *Haplorchis yokogawai* | 3 (3.1) | 3 (3.7) | 0 | NA | NA |
| Cestodes | | | | | |
| *Taenia saginata* | 18 (18.6) | 18 (22.0) | 0 | 4.10 | 0.043 |
| Other *Taenia* spp. | 5 (5.2) | 4 (4.9) | 1 (6.7) | 0.08 | 0.782 |

NA: not applicable.

severely sick (6 with severe kidney stone disease and 1 each with liver tumour, ascites or liver cirrhosis) or pregnant ($n = 1$). Hence, 97 individuals were purged, 82 (84.5%) in the community-based survey and 15 (15.5%) in the hospitals. Lao Loum was the main ethnic group and patients were aged 15—75 years. The illiteracy rate among females in the community survey was significantly higher than among males (45.0% vs. 34.2%; $\chi^2 = 4.09$, $P = 0.043$), whilst only one of the participants was illiterate in the hospital study. Subsistence farming was the main occupation of study participants both in the community and the hospital-based surveys (89.0% and 67.7%, respectively).

### 3.4. Number and species of worms collected after purgation

Table 3 shows the species-specific prevalence of intestinal parasites recovered from study participants after purgation. Adult *O. viverrini* flukes were diagnosed in 95 individuals (97.9%). Additionally, adult worms of six different species of MIF were identified, with *Haplorchis taichui* being the most common (78.4%). The prevalences of *Phaneropsolus bonnei* and *Prosthodendrium molenkampi* were 22.7% and 14.4%, respectively. Significantly higher prevalences were observed in the community-based study compared with hospitalised patients for *O. viverrini* (100% vs. 86.7%; $P = 0.001$), *H. taichui* (84.1% vs. 46.7%; $P = 0.001$) and *Taenia saginata* (22.0% vs. 0%; $P = 0.043$).

Seventy-nine individuals (81.4%) harboured *O. viverrini* and at least one species of MIF concurrently. Conversely, single infections both with *O. viverrini* and MIF were rare, with prevalences of 16.5% and 2.1%, respectively. Two of the parasites were significantly associated with age group, namely *P. molenkampi* ($\chi^2 = 10.63$, $P = 0.031$) and *P. bonnei* ($\chi^2 = 9.18$, $P = 0.050$), with a peak prevalence in the 36—45 years age group. Figure 3 shows species-specific age—prevalence curves of adult flukes following purgation.

Table 4 summarises the total number of flukes collected from the 97 purged individuals, including arithmetic mean counts and 95% confidence intervals (CIs). Very high total counts were recorded for *O. viverrini* (17 755; community 14 802, hospital 2953) and *H. taichui* (15 555; community 14 530, hospital 1025). The mean fluke count for *O. viverrini* was 186 (community 182, hospital 206) and the mean count for *H. taichui* was 207 (community 214, hospital 146).

Figure 4 depicts the association between the number of adult *O. viverrini* flukes recovered after purgation and the infection intensity as expressed by epg upon microscopic stool examination using FECT. Linear regression analysis showed a significant positive association, with a regression

**Table 4** Number (including arithmetic mean and 95% CI) of trematodes discovered after purgation of 97 individuals in Lao PDR.

| Parasite | Overall | Community | Mean (95% CI) | Hospital | Mean (95% CI) |
|---|---|---|---|---|---|
| Liver flukes | | | | | |
| *Opisthorchis viverrini* | 17 755 | 14 802 | 182 (117—246) | 2953 | 206 (53—359) |
| Intestinal flukes | | | | | |
| *Haplorchis taichui* | 15 555 | 14 530 | 214 (101—326) | 1025 | 146 (54—239) |
| *Phaneropsolus bonnei* | 910 | 735 | 37 (6—67) | 175 | 88 (13—206) |
| *Prosthodendrium molenkampi* | 562 | 482 | 37 (4—70) | 80 | SN |
| *Haplorchis yokogawai* | 154 | 154 | SN | 0 | 0 |
| *Haplorchis pumilio* | 108 | 108 | 22 (2—41) | 0 | 0 |
| *Echinochasmus japonicus* | 10 | 10 | 3 (1—6) | 0 | 0 |

SN: small number.

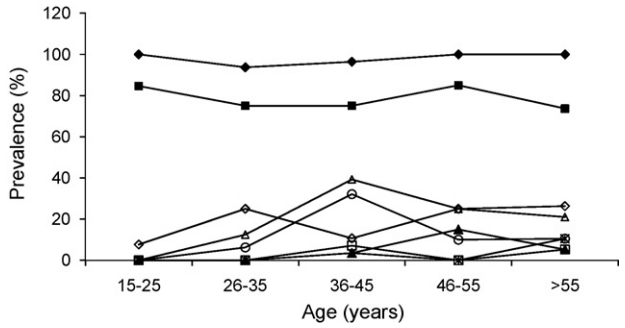


**Figure 3** Age—prevalence curves for *Opisthorchis viverrini* (♦), *Haplorchis taichui* (■), *Echinochasmus japonicus* (□), *Taenia saginata* (◊), *Phaneropsolus bonnei* (△), *Prosthodendrium molenkampi* (○), *Haplorchis pumilio* (▲) and *Haplorchis yokogawai* (*) detected in 97 purged individuals in Lao PDR.

equation of $y = 0.98 + 0.92x$ ($r = 0.54$; $P < 0.001$). No association between adult worms recovered and eggs counts was found for MIFs ($r = 0.22$, $P = 0.093$). Figure 5 depicts the association between adult worm counts of *O. viverrini* and egg counts per worm. A significant negative correlation was found ($r = -0.40$, $P < 0.001$), with a linear regression equation of $y = 2.18 - 0.36x$ ($P < 0.001$).

### 3.5. Diagnostic performance of FECT

The validity of FECT for diagnosis of *O. viverrini* and MIFs was assessed considering purgation as the diagnostic 'gold standard'. The sensitivity of FECT for the discovery of *O. viverrini* and MIF eggs was 96.8% (92/95) and 85.0% (68/80), respectively. The specificity and positive predictive value (PPV) for diagnosing *O. viverrini* eggs were 100.0% (92/92), regardless of the method. The specificity and PPV of FECT for MIF diagnosis were 70.6% (12/17) and 93.2% (68/73), respectively. For both parasites, the negative predictive value of FECT was low; 60.0% (3/5) in the case of *O. viverrini* and 50.0% (12/24) for MIFs.

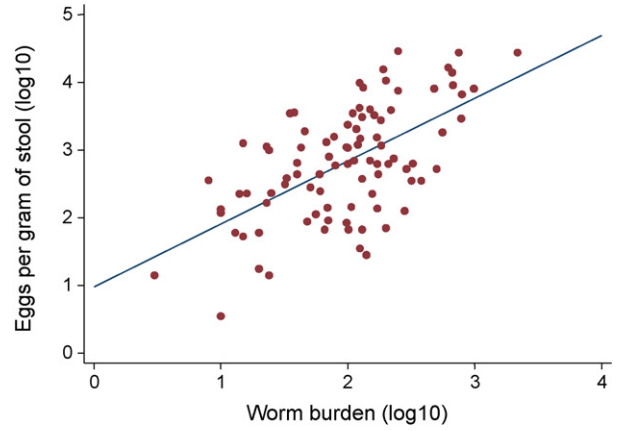


**Figure 4** Logarithmic transformation of the association between the number of *Opisthorchis viverrini* flukes recovered after purgation ($n = 97$) and egg counts by formalin ethyl-acetate concentration technique.

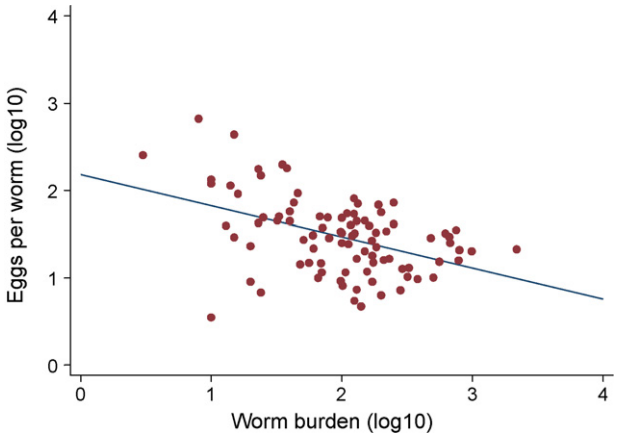


**Figure 5** Logarithmic transformation of the association between the number of *Opisthorchis viverrini* flukes recovered after purgation ($n = 97$) and eggs per worm by formalin ethyl-acetate concentration technique.

## 4. Discussion

Data obtained from 232 Lao individuals aged ≥15 years who complained of hepatobiliary or intestinal symptoms confirm that multiparasitism is the norm rather than the exception. In fact, more than three-quarter of the participants harboured at least two helminth species concurrently, with *O. viverrini* and MIFs being the most common trematodes encountered. A rigorous diagnostic approach was employed, with three Kato-Katz thick smears performed on consecutive stool specimens, supplemented with FECT on one of the specimens. Previous studies have shown that multiple Kato-Katz thick smears plus other diagnostic methods are mandatory to achieve a high diagnostic sensitivity.[21–23] In the present study, only 15 individuals (6.5%) were diagnosed as free of any intestinal parasite. It is conceivable that the actual number of parasite-free individuals is even lower, as some light infections might have been missed despite the rigorous diagnostic approach. Besides the liver fluke *O. viverrini* and various kinds of intestinal flukes, hookworm infections were also highly prevalent. Interestingly, *S. stercoralis*, arguably the most neglected of the soil-transmitted helminths,[23,24] was more prevalent than *T. trichuris* and *A. lumbricoides*. Purgation of a subsample of individuals allowed identification of parasites at species level, with MIFs and *H. taichui* found at the highest frequency.

Our data underscore that multiparasitism is very common in Lao PDR and these findings support observations from neighbouring countries such as Vietnam[25–27] and from other parts of the developing world.[21,22,28–30] Hence, our results and those from other groups who worked in Lao PDR[7,8] call for concerted action to remedy the issue of multiparasitism. Of particular public health relevance are our results in relation to the diversity of trematodes identified. In the purgation of 97 patients, as many as seven different trematode species were identified. *O. viverrini* was the most abundant fluke, followed by *H. taichui*. Another five intestinal trematodes were recorded (*P. bonnei*, *Haplorchis yokogawai*, *P. molenkampi*, *Haplorchis pumilio* and *Echinochasmus japonicas*) although at significantly lower prevalences, which is consistent with previous reports from other parts of Lao

PDR.[13,31] A number of the identified human trematodes have eggs of similar size and shape.[17] Species differentiation is therefore difficult with simple coprologic diagnostic tools such as direct faecal smears or the widely used Kato-Katz thick smear examination. However, differentiation of MIFs from *O. viverrini* is of considerable public health importance as the latter fluke provokes a range of hepatobiliary diseases and is a major risk factor for cholangiocarcinoma.[9,32] Hence, monitoring of interventions that are targeted against *O. viverrini* require diagnostic techniques that are capable of differentiating between *O. viverrini* and MIFs.

Our analysis showed that *O. viverrini* worm burden is highly significantly associated with the egg counts in stool samples. Very similar linear regression lines were described in Thailand.[33,34] Hence, we can confirm that egg counts are a valid proxy measure for intensity of infection with *O. viverrini*. In addition, similar to Elkins et al.,[33] we also found a decreasing fecundity of *O. viverrini* with increasing worm burden, a phenomenon that may reflect density-dependent constraints on fecundity.[34,35]

Our validation of a commonly employed faecal concentration technique with results of the purging examination, the latter serving as a diagnostic 'gold standard', showed high sensitivity and specificity for diagnosis both of *O. viverrini* and MIFs. Our findings therefore support the use of FECT in future studies emphasising parasite species-specific diagnosis. Repeated stool examinations have been shown to improve significantly the diagnostic accuracy for parasitic infections.[22,24,36–38] It follows that FECT performed on multiple stool samples holds promise for accurate and species-specific diagnosis. However, such an approach is time consuming and is likely to compromise compliance, as study participants are reluctant to provide multiple stool specimens. Alternatively, species-specific PCR-based techniques have been developed.[39–41] However, PCR methods are less suitable for large-scale community-based investigations as they are costly and still of limited direct applicability under field conditions.

It is important to note that dishes based on raw or insufficiently cooked fish, other aquatic products and meat are frequently consumed in Lao PDR and other Southeast Asian countries. The common habit of raw or undercooked fish consumption is a key factor in the transmission of trematode infection, which in turn explains the high prevalence and infection intensity of *O. viverrini*; indeed, over one-half of the subjects examined harboured a moderate or heavy infection with *O. viverrini*.

Interestingly, we also found two MIF species that are transmitted by consumption of raw naiads, i.e. *P. bonnei* and *P. molenkampi*. Both species were actually diagnosed quite frequently (22.7% and 14.4%, respectively). In the 1970 s, the first cases of human infections with these trematodes were described in the Udon Thani area of northeastern Thailand, where communities have similar alimentary habits as in Lao PDR.[42] Furthermore, in three patients living in Khamsida village, a rare Echinostomatidae fluke was diagnosed, namely *E. japonicus*. To our knowledge, this is the first report of *E. japonicus* from Lao PDR and details will be presented elsewhere.

Of clinical and public health importance is the fact that virtually all patients included in the study were infected with trematodes, with more than three-quarters harbouring *O. viverrini* and MIFs concurrently. Conversely, only a few patients had a single-species infection (*O. viverrini* 16.5%, MIF 2.1%). Although it is acknowledged that polyparasitism may negatively impact on health and well-being, new research is needed to deepen our understanding of the underlying mechanisms and how to measure improvements following control measures at the individual and population level. Further investigations coupled with rigorous monitoring of control interventions are urgently needed to further our knowledge and to provide a rationale for evidence-based interventions.

**Authors' contributions:** SS and PO designed the study; SS, YV, MV and PO collected field data; SS and OR collected hospital data; SS and ST identified parasites in the laboratory; SS analysed data and drafted the manuscript; PO, JU and KA contributed to data analysis and revised the manuscript; KA held overall responsibility for data collection. All authors read and approved the final manuscript. SS and PO are guarantors of the paper.

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