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RESEARCH ARTICLE

Molecular identification of *Opisthorchis viverrini* among the northeastern Cambodian population by internal transcribed spacer 2 based polymerase chain reaction

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ABSTRACT

The southeast Asian fluke *Opisthorchis viverrini* remains endemic, particularly in Thailand, Lao PDR, Cambodia, Vietnam, and Myanmar. However, there is a lack of data on the prevalence of liver fluke infection in Kratie Province in northeastern Cambodia. The present study aimed to detect *O. viverrini* DNA in fecal specimens by using the internal transcribed spacer 2 (ITS2) region of ribosomal DNA (rDNA) based on polymerase chain reaction (PCR). The prevalence and percentage of *O. viverrini* infection were described by data analysis. Bivariate binary logistic regression analysis was used to look at the related prevalence of *O. viverrini* infection. A total of 6.89% from 377 fecal samples were found positive of *O. viverrini* DNA. The prevalence of *O. viverrini* infection was found to be higher in men (8.92%) than in women (5.45%), and to be associated more frequently with younger age groups (13.40%), illiteracy (8.74%), participation in other careers (non-specific occupations) (11.63%), and residence in the Trapaing Srae village (9.94%) of the Snuol district, Kratie Province. Age groups under 20 years old were significantly linked with *O. viverrini* infection, with ORadj=0.601, 95% CI=0.410-0.882, p=0.009 and significant value established at (P<0.05). This study demonstrates that *O. viverrini* infection is distributed in rural areas located near freshwater reservoirs. Therefore, active surveillance, clinical examination of association with hepatobiliary, cholangiocarcinoma, and health education are needed.

Keywords: *Opisthorchis viverrine*; cholangiocarcinoma; PCR; ITS2; Cambodia.

INTRODUCTION

Human liver fluke, *Opisthorchis viverrini*, is still a serious public health concern globally and is often contracted in nations in Southeast Asia's Greater Mekong subregion, including Thailand, Lao PDR, Cambodia, Vietnam, and Myanmar (Sripa *et al*., 2010; Aung *et al*., 2017). The diversity riverside of Mekong subregion in multiple countries in Southeast Asia is obviously known highly endemic with *O. viverrini* infection (Radomyos *et al*., 1998; Khieu *et al*., 2019). There are 3 major species of liver flukes, *O. viverrini*, *opisthorchis felineus* (*O. felineus*), and Clonorchis sinensis (*C. sinensis*) has been recognized by World Health Organization (WHO), as foodborne trematodiases, including liver, lung, and intestinal flukes that trigger liver diseases in human and animals. Cholangiocarcinoma (CCA), a condition caused by the infection of these flukes, is classified as a Group 1 carcinogen (WHO, 2020). The adult worm of *O. viverrini* has been reported as a factor involving in hepatobiliary diseases associated with CCA development (Fried & Abruzzi, 2010).

The life cycle of liver flukes is similarly, and they need two intermediate hosts such as *Bithynia* snails (first intermediate) to develop into cercaria and cyprinoid fishes (second intermediate) into metacercariae. The mammalian (definitive hosts) including human and domestic animal become infected by consumption of raw or undercooked freshwater fishes containing metacercariae that encyst in fish flesh, which is a main route of infection. After ingestion, hermaphroditic worm ascending to hepatobiliary duct and then grow in the bile duct as adult flukes (Thu *et al*., 2007). Two small human liver flukes frequently described, among the pathogens related to hepatobiliary tract infection in southeast Asia and China (Keiser & Utzinger, 2009; Sripa *et al*., 2010). The symptom in the definitive hosts (human) is showed as clinical manifestation including fever, anorexia, weight loss, fatigue, yellow sclera, and jaundice, however infection is attributed into cholangitis in acute stage results in bile duct obstruction and inflammation. In severe pathological symptoms caused of *O. viverrini* and *C. sinensis* were frequently induced in

relapsing cholangitis, periductal fibrosis, cholecystitis, liver cirrhosis, cholelithiasis, and CCA (Sripa *et al*., 2021; Chai & Jung, 2022).

The prevalent of liver flukes infection to human was reported over 600 million people at high risk of infection with this neglected trematode (Keiser & Utzinger, 2005). The estimation prevalence among the population of *O. viverrini* infection in Southeast Asia based on various geographical region in some countries including Thailand (6.71 million), Lao PDR (2.45 million), Vietnam (2.07 million), and (1 million) Cambodia (Zhao *et al*., 2021).

The National Center for Malaria Control, Parasitology and Entomology (CNM) departments and the Ministry of Health (MOH) of Cambodia was interested researching on epidemiological survey of parasitology in the endemic province after the first publishing of *O. viverrini* infection in Cambodia in PubMed database by Korean researcher team, in 2002 (Lee *et al*., 2002). The initial investigation into *O. viverrini* infection has been started since 2006 in two villages in the Prey Kabas district, Takeo Province (Miyamoto *et al*., 2014). The National Helminth Control Program (NHCP) launched to conduct of field survey of *O. viverrini* infection that covered almost countrywide by collaboration with oversea researcher team especially Korean researchers. According to Vireak Khieu in 2019, the geographic distribution of *O. viverrini* infection covers more than 20% in the north across the center to southern province. The prevalence of egg positive rate among the residence was found in central and southeastern province, began from Kampong Thom (34.8%), Kampong Cham (24.0%), Kandal (20.2%), and especially in three villages of Takeo Province range from 46.4 to 50.6%, respectively (Yong *et al*., 2012, 2014; Khieu *et al*., 2019).

Regarding to the northeastern Cambodia, the province geological boundary of Kratie Province bordering to Stueng Treng, Kampong Thom, Kampong Cham, Tboung Khmom, Mondulkiri Province, and South Vietnam. The Greater Mekong River Subregion extending flow a cross 6 province in Cambodia including Stung Treng, Kratie, Tboung Khmom, Kampong Cham, Kandal, and Prey Veng Province (National Institute of Statistic, 2020). In previous study was found a highest prevalence (4.6%) of *O. viverrini* infection in Kratie Province (Sohn *et al*., 2012). The very recently study was reported that the standing of *O. viverrini* and/or minute intestinal flukes (MIFs) infection among population from 10 villages in Preah Vihear and Stung Treng Province with the average prevalence 59.8% of egg-positive cases. Thus, liver flukes, *O. viverrini* infection is an emerging disease in the northeastern cross the central to southern provinces (Jung *et al*., 2023).

In addition, the morphologies of *O. viverrini*-egg through fecal sample under a light microscope based on the parasitological techniques is not well differentiated (Kaewkes, 2003; Sithithaworn *et al*., 2007). However, polymerase chain reaction (PCR) based on the internal transcribed spacer 2 (ITS2) has higher specificity and sensitivity to identify the *O. viverrini* DNA through fecal samples (Wongratanacheewin *et al*., 2002). Therefore, PCR method was performed to amplify *O. viverrini* DNA with 33 of positive cases in Kampong Thom and Kampong Cham Provinces (Miyamoto *et al*., 2014).

The present study aimed to identify the *O. viverrini* DNA in fecal sample among residents in the Snuol district, Kratie Province, northeastern Cambodia, utilizing ITS2 gene targeting in ribosomal DNA (rDNA) based on. The prevalence of *O. viverrini* infection among the population from 5 village in the Snuol district, Kratie Province, which was conducted the study at the parasitic disease research center (PDRC), Institute of Medicine, Suranaree University of Technology (SUT), Thailand.

MATERIALS AND METHODS

Study areas and samples

The sample size was calculated by the equation: $n=[N(Za/2)2P(1–P)]/[e^2(N–1)+(Za/2)2P(1–P)]$, which is represented by N= the sample size or population, P=prevalence or proportion of infected individuals, Za/2=normal deviation for two-tailed alternative hypothesis at a level of significance of 1.96, and e=precision margin of error (5% or 0.05) (Ngrenngarmlert *et al*., 2012). The sample size was set at 377 individuals. The study design of accept the fecal samples was using a cross-sectional design for collecting fecal samples as a simple random sampling technique. This study was conducted using fecal sample from Kratie Province, northeastern Cambodia. Kratie Province is one among five provinces located in the Mekong River Basin in Cambodia. The target villages, including Trapaing Srae, Chrab, Cheung Khie, Pravanh, and Cheung Khlu villages were in the Pithnu subdistrict, Snuol district, and Kratie Province, as mapped in red label (Figure 1). Fecal samples were collected beginning in 2019 and stored at 4°C until examination. This study specimens were approved by the Human Ethics Committee from the health office in Nakhon Ratchasima Province (EC: NRPH013) and ensuring the ethical regulation by the Ministry of Health of Cambodia. Additionally, the BioEthic for conducting in laboratory experiment in PDRC was approved by BioEthic committee number (IBC-65-05) Institute of Research and Development (IRC), SUT. On the other hand, signed informed consent for participation was obtained, with a witness signing for young participants. Therefore, young participants used a fingerprint instead of their name if they were unable to write by themselves.

Genomic DNA extraction

The *O. viverrini* DNA was extracted from fecal specimens using a feces QIAamp1 DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Briefly, 180 mg of stool in 2 mL was added to 1 mL of inhibit EX buffer in a microcentrifuge tube. The stool sample was homogenized by heating at 95°C for 5 minutes (min) and centrifuged at 14,000 rpm for 1 min. Then, 200 µL of supernatant was added to 15 µL proteinase K in a tube, and 200 µL of AL buffer was added. The mixed solution was incubated at 70°C for 10 min and lysed with 95% ethanol (200 µL). The supernatant (600 µL; lysate) was transferred to a spin column and centrifuged for 1 min, which 500 µL of AW1 and AW2 buffer were consecutively added and centrifuged. To elute DNA, 25 µL buffer ATE was added, incubated, and centrifuged for 1 min. Finally, the extracted DNA gene was stored at -20°C until use.

Amplification of the *O. viverrini* ITS2

The fecal specimens were separated into 2 mL microcentrifuge tubes for DNA extraction for molecular identification. Five villages in the Pithnu subdistrict, Snuol district, Kratie Province, were selected. A total of 377 fecal samples were used with initial primers, such as ITS2. The ITS2 was amplified using primers Ov-6F 5'-GTT CCA GGT GAG TCT CTC TA-3' and Ov-6R 5'-TGA ACA AAC GAG AGA TTC A-3' (Wongratanacheewin *et al*., 2002), with a product size of 330 base pairs (bp). The PCR-ITS2 reaction was conducted in a total volume of 25 µL containing PCR buffer, 16.8 µL of H_2O, 2.5 µL of 10x Taq buffer, 1 µL of dNTP, 1 µL of IST2-OvF, 1 µL of ITS2-OvR, 0.2 µL of Taq DNA polymerase, 1.5 µL of $MgCl_2$, and 1 µL of DNA template. The initial denaturation temperature was 94 °C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C for 45 sec (Duenngai *et al*., 2008). The

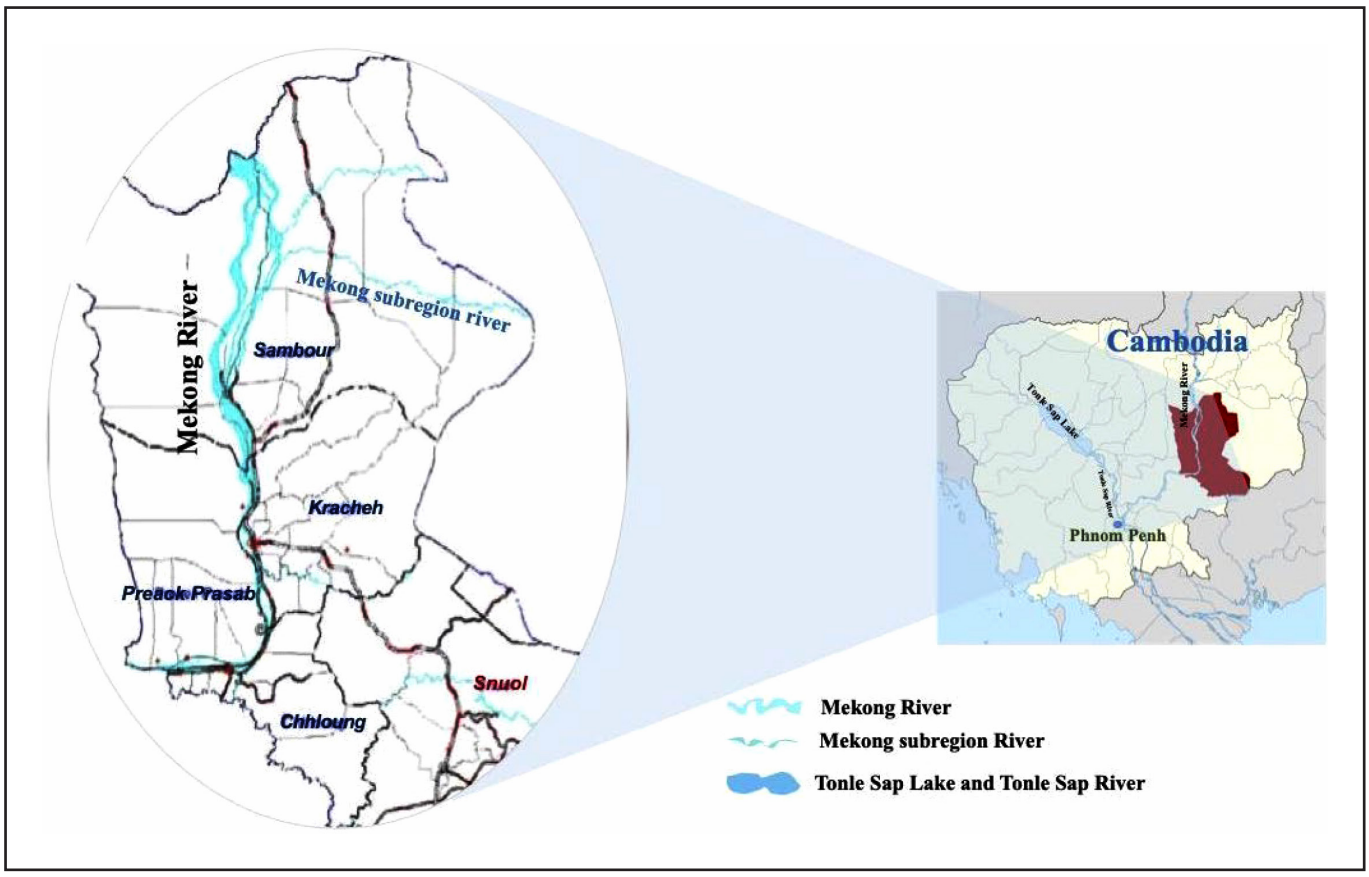

Figure 1. Map of Snuol district (red labeled), Kratie Province, Cambodia. The Greater Mekong River crosses Kratie Province (Modified from a provincial map, 2010).

PCR products were amplified using the G-STORM^{TM} GS482 thermal cycler. The PCR-ITS2 products were assessed by 1.5% agarose gel electrophoresis with 30 mL of 1x TAE buffer. The DNA marker, DNA loading, maestrosafe nucleic acid stain (MNAS), and PCR products were mixed, and gel electrophoresis was carried out at 100 V at room temperature for approximately 30 min. In the final step, the agarose gel was visualized using a molecular image® Gel Doc TMXR Imaging System (Bio-Rad). The positive amplicons were sent for sequencing.

Sanger sequencing of the ITS2 region

Positive amplicons of the *O. viverrini* ITS2 region were purified and sequenced by QIAGENE company (Bangkok, Thailand). The PCR amplicons each 10 µL PCR tube were labeled by an ID code for product identity. The sequence results were obtained as Fasta file accession numbers for searching paired with the sequence in NCBI BLAST in the GenBank database.

Statistical analysis

Analysis was performed with SPSS version 23.0 software (SPSS Inc., Chicago, USA) for Windows. Frequencies are described by crosstabs and chi-square tests, including the association between the prevalence of *O. viverrini* infection and variables analyzed by bivariate binary logistic regression, which were expressed as the percentage and 95% confidence interval (95% CI); $p<0.05$ was considered statistically significant. A multivariable regression model is used to demonstrate a dependent variable more than one that has relationship with one to another variable for selecting from a significant bivariate model.

RESULTS

The molecular detection of *O. viverrini* ITS2

A total of 377 fecal samples were used to extract DNA for the purpose of identifying *O. viverrini* using ITS2 gene-based PCR. The *O. viverrini* ITS2 gene was detected in 26/377 (6.89%) cases combined with a specific amplicon about 330 bp for both individual diagnoses using the above mentioned forward and reverse primers. The amplified bands were determined as shown in (Figure 2). The 330-bp PCR product of the *O. viverrini* ITS2 gene was confirmed by DNA sequencing. The 26 positive results matched the *O. viverrini* genomic 333 bp mRNA in GenBank sequences (Sermswan *et al*., 1991) using BLAST program of the NCBI accession number S80278.1, which was represented recognizing as similarly, respectively. The initial reference sequence was selected from the positive detected PCR-product of the specific primer ITS2 for amplification of *O. viverrini* DNA targeting in ribosomal DNA. The bilateral parallel numeric descended data are recorded in GenBank. Therefore, the alignment part of *O. viverrini* sequence with accession numbers is shown as the letter representative of the different regions (Figure 3).

The association prevalence of *O. viverrini* infection

Demographic characteristics, including sex, age group, education, occupation, and location, in association with *O. viverrini* infection in Kratie Province, Cambodia were analyzed using the regression binary logistic data analysis (Table 1). A total of 377 fecal specimens in males were found (n=14, 8.92%), which was higher than in females (n=12, 5.45%). As a result, the most common *O. viverrini* infection was in the

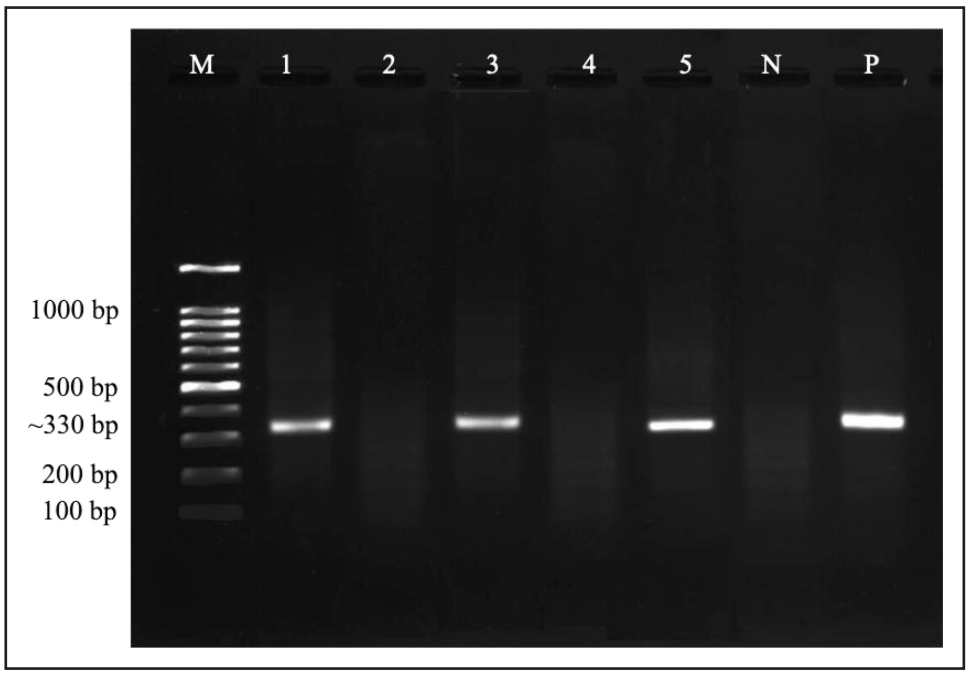

Figure 2. *O. viverrini* was amplified based on internal transcribed spacer 2 (ITS2) and visualized under 1.5% agarose gel electrophoresis. M-marker or ladder 100 bp; N-negative control; P-positive control; the expected product is about 330 bp. Lanes 1, 3, and 5 the sample products are showing of positive bands, whereas lanes 2 and 4 are showing of negative bands.

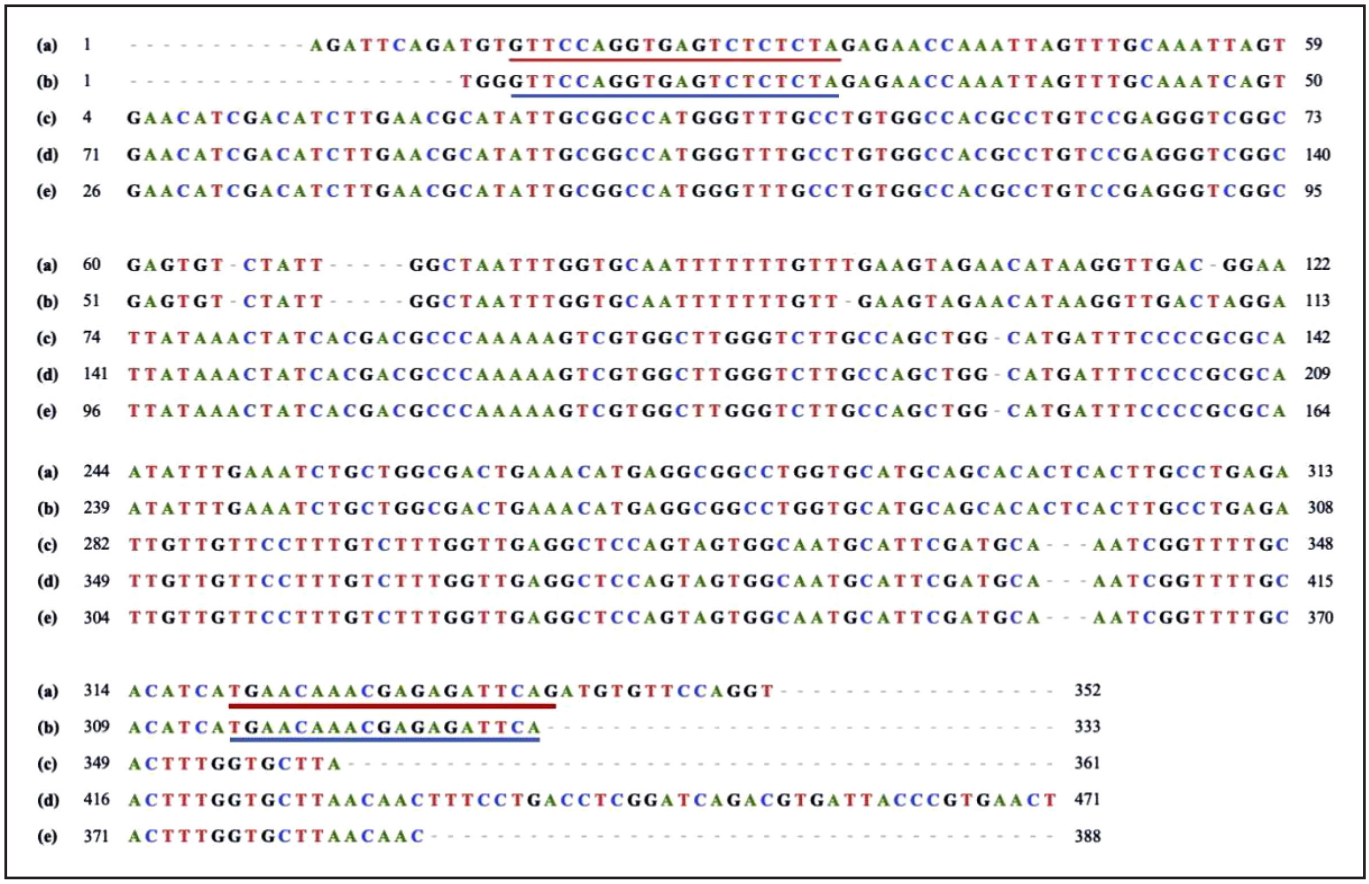

Figure 3. Graphic view alignment of the *O. viverrini* ITS2 gene product with the sample sequence. (a) A positive fecal specimen sequence from Cambodia (accession number: CK0096.1) was amplified by ITS2-PCR with a specific amplicon about 330 bp, which was matched with (b) *Opisthorchis viverrini* sequence of the primer product (accession number: S80278.1) which is ITS2-PCR with amplicon about 333 bp. (c) *O. viverrini* sequence of Lao PDR from GenBank (accession number: HQ328549), (d) *O. viverrini* sequence of Thailand (accession number: AY584735), and (e) *O. viverrini* sequence of Vietnam (accession number: KT726408). The dashes in the graphic view indicated the absence of residues, and *O. viverrini* species existing simulation residues. The forward (top) and reverse (bottom) of template sequences are indicated as red lines and the primer product sequence is indicated as blue lines.

Table 1. The prevalence of *O. viverrini* infection using ITS2-PCR (n=377) association with characteristics

Characteristic	n (%)	*O. viverrini* n (%)	OR_{cru} (95% CI)	*P*-value	OR_{adj} (95% CI)	*P*-value
Sex						
Male	157 (41.64)	14 (8.92)	0.589 (0.265–1.311)	0.195	0.626 (0.269–1.457)	0.277
Female	220 (58.36)	12 (5.45)				
Age groups						
< 20 years	97 (25.73)	13 (13.40)				
21-30 years	104 (27.59)	8 (7.69)				
31-40 years	70 (18.57)	2 (2.86)	0.569 (0.388–0.836)	0.004*	0.601 (0.410–0.882)	0.009*
41-50 years	58 (15.38)	2 (3.45)				
>51 years	48 (12.73)	1 (2.08)				
Education level						
Illiteracy	103 (27.32)	9 (8.74)				
Primary	133 (35.28)	11 (8.27)	0.696 (0.422–1.148)	0.156	0.765 (0.451–1.299)	0.322
Secondary and higher	141 (37.40)	6 (4.26)				
Occupation						
Employee	41 (10.88)	2 (4.88)				
Farmer	144 (38.20)	12 (8.33)				
Shopkeeper	68 (18.04)	4 (5.88)	1.052 (0.810–1.368)	0.703	1.022 (0.776–1.347)	0.875
Housewife	62 (16.45)	2 (3.23)				
Government - officer	19 (5.04)	1 (5.26)				
Others	43 (11.41)	5 (11.63)				
Location						
Trapaing Srae	161 (42.71)	16 (9.94)				
Chrab	63 (16.71)	1 (1.59)				
Cheung Khie	52 (13.79)	1 (1.92)	0.900 (0.681–1.189)	0.460	0.934 (0.701–1.243)	0.638
Pravanh	42 (11.14)	4 (9.52)				
Cheung Khlue	59 (15.65)	4 (6.78)				

*Logistic regression Binary test, significantly different *P*-value<0.05.

age group less than 20 years old (*n*=13, 13.40%) among all variables with a crude odds ratio (OR_{cru})=0.569, 95% CI=0.388-0.836, *P*=004 and an adjusted odds ratio (OR_{adj})=0.601, 95% CI=0.410-0.882, *P*=0.009 indicating a significantly high risk of *O. viverrini* infection. The following age group was 21-30 year (*n*=8, 7.69%), 31-40 year (*n*=2, 2.86%), 41-50 (*n*=2, 3.45%), and age group over 50 year (*n*=1, 2.08%), respectively. The variable in education level was more frequently infection among illiteracy (n=9, 8.74%) and followed by primary school kids (*n*=11, 8.27%), the secondary and higher were (*n*=6, 4.26%), no remarkable as significant. For occupation the others career (non-specific occupation) (*n*=5, 11.63%) were notably more frequently infected compared with specific career including employee (*n*=2, 4.88%), farmer (*n*=12, 8.34%), shopkeeper (*n*=4, 5.88%), housewife (*n*=2, 3.23%), and government officer (*n*=1, 5.26%). Regarding location infection was more common in Trapaing Srae village (*n*=16, 9.94%) than in other villages, however there was not significantly.

DISCUSSION

The genus of *O. viverrini* specie has been known in the Mekong River basin. In previous study was reported of molecular characteristics of recovery *O. viverrini* in the patient using the NADH dehydrogenase subunit 1 (*nad*1) among the population in Kratie Province (Sohn *et al*., 2012). In the molecular marker including the ITS2, cytochrome *c* oxidase subunit 1 (*cox*1), and *nad*1 sequence were most used to compare of the nucleotide difference between Opisthorchis spp. and *C. sinensis* (Thaenkham *et al*., 2011). On other hand, the mitochondrial DNA (mtDNA) genes marker is extending to identify genetic diversity of liver flukes' sub-type in different regions along the Mekong River including Thailand, Lao PDR, Cambodia, and Vietnam (Thaenkham *et al*., 2010). However, *nad*1-based PCR was utilized to reveal homologous nucleotide sequences (97-99%). ITS2 sequenced was more specificity than cytochrome c oxidase subunit 1 (*cox*1), with 0.86% and 3.03% nucleotide differences between *O. viverrini* and *Opisthorchis lobatus,* respectively (Thaenkham *et al*., 2011). The molecular methods were used to study in the form of identifying the genus population and sub-type of adult flukes, *O. viverrini* spp. by some researchers in Cambodia (Thaenkham *et al*., 2010; Sohn *et al*., 2012).

This study is demonstrated of molecular identification of *O. viverrini* rDNA in northeastern Cambodia using ITS2-PCR and graphic alignment of nucleotide sequences between the product primers sequences and samples sequences by using references sequence in GenBank data base to confirm the molecular alignment. A graphic view of alignment was put together with the nucleotide sequence representing the *O. viverrini* ITS2 gene that specified the matches of the product and sample sequence found in the human host. These DNA sequences were recommended for designate alignment to differentiate molecular diagnosis of mutation genes. The graphic alignment result indicated of the most similarly of *O. viverrini* sequence between the product and sample sequence were most likely paired, respectively.

A meta-analysis statical was conducted an analysis of *O. viverrini* infection with the prevalence over 20% among the population in northern to southern province in Cambodia (Khieu *et al*., 2019). The very recently study was conducted to investigate the ranking of *O. viverrini* infection in the Preah Vihear and Stueng Treng Provinces through fecal samples. The highest prevalence was (59.8%) of egg-positive cases among population in theses provinces that found to be highest increasing ever reported in Cambodia (Jung *et al*., 2023). However, according to Korean researcher team was reported about

a nationwide survey by highest prevalence of *O. viverrini* infection in Kampong Cham (24.0%), Takeo (23.8%), and lower prevalence followed by Preah Vihear (2.7%), Stung Treng (2.5%), Kratie (3.4%), and (2.8%) Ratanakiri Province, respectively. Another study of riparian population was infected by the *O. viverrini* in the Prey Kabas district, Takeo Province with higher prevalence (47.5%) was reported (Yong *et al*., 2012). A couple years latter there was clarified of *O. viverrini* infection distribution through the Provinces along the Mekong River basin in Cambodia was found higher prevalence in Kampong Thom (34.8%), which followed by Kampong Cham (33.1%), Takeo (21.4%), and (18.7%) Kandal Province (Miyamoto *et al*., 2014). The prevalence (4.6%) of *O. viverrini* egg-positive cases was reported in Kratie Province (Sohn *et al*., 2012). However, there was revealed of *O. viverrini* infection with higher prevalence (5.36%) among population in the Snuol district, Kratie Province (La *et al*., 2022). Therefore, in this study the prevalence of *O. viverrini* infection which was identified among the population in the Snuol district, is highest at any point in Kratie Province.

It has long been known by epidemiologists that males of vertebrate species, including humans, typically exhibit higher rates of illness and parasitism than females (Bundy, 1988; Zuk, 1990). Ecological and physiological mechanisms are frequently used to categorize the origins of sex biases in parasitism rates. Ecological mechanisms include sex variations in behavior, nutrition composition, and body size (Zuk, 1996). Due to various male social traditions, such as the frequent ingestion of raw or undercooked freshwater fish with alcohol in social settings, males in South Korea showed a higher incidence of *C. sinensis* infection than females, whereas in China an indigenous species the comparable trends were seen (Rim *et al*., 1990; Rim et al., 2003). On the other hand, housewives, or females, were shown to have the highest incidence of *O. felineus* infection in Russia (Rim *et al*., 2003). However, it has been noted that there is a male preponderance or no discernible sex difference in Thailand, where *O. viverrini* is common (Rim *et al*., 2003; Kobayashi *et al*., 2000; Wattanawong *et al*., 2021). In Cambodia, a male preponderance was reported in Takeo province (Yong *et al*., 2012). In the present study, no significant sex difference was noted. One of the reasons may be that the major source of infection in the surveyed areas is traditional fish dishes popularly consumed by the people regardless of gender, such as '*plea tre*' (fish salad), '*plea tre chou*' (sour fish salad), and '*ma'am*' (fermented fish, kept for 2–5 days) (Jung *et al*., 2023).

In fact, the parasites are reproduced after transmitted within the host and they are adapted from environment over time and gradually increasing with the host age. Numerous empirical research has documented age-intensity, showing either a persistent rise in parasite load or a gradual elevated of parasite burden with age (Hudson & Dobson, 1995). *O. viverrini* infection was documented in infant (6 months) in a rural community, Lao PDR; however, infection rates in young age were lower than those in elderly, despite a significant risk to all age groups. (Kobayashi *et al*., 2000), and similar to the age-prevalence observation in Thailand (Sithithaworn & Haswell-Elkins, 2003; Kaewpitoon *et al*., 2008). In 2013, there was countrywide research of the students that funded by USAID in Cambodia to determine the prevalence infection caused by *O. viverrini* in children was found (4.10%) (Khieu *et al*., 2019; Lee *et al*., 2002). However, one of recently investigation of *O. viverrini* infection in Preah Vihear and Stung Treng Province was prevalence higher by the age group 30-39 years and followed by age group 40-49, 50-59, and over 60 years, whereas the prevalence was lower among children under 9 years, age group 10-19, and 20-29 years (Jung *et al*., 2023). Their finding was similar trended of liver fluke infections that have been seen in other nations, including *O. viverrini* in Lao PDR and Thailand (Forrer, 2012; Suwannahitatorn *et al*., 2019; Pengput, 2020), *O. felineus* in Russia (Rim *et al*., 2003), and *C. sinensis* in South Korea (Rim *et al*., 1990). Additionally, the riverside population in Cambodia's Takeo province showed a nearly same age-prevalence trend for *O. viverrini* infection (Khieu *et al*., 2019; Yong *et al*., 2012). Thus, in this present study was more frequently higher prevalence in young age groups less than 20 years was significantly and the lower prevalence followed by age group 21-30 years.

Moreover, in this study, the illiteracy rate was greater among people with higher levels of education. Poor literacy is strongly correlated with intestinal parasite infection, according to certain research. *O. viverrini* infection prevalence among the age groups was associated with illiteracy (8.74%) levels of education. The risk factor for the illiteracy rate was present (Nematian *et al*., 2004). According to several research, parasitoses are correlated with illiteracy, a lack of latrines causing diarrhea, a lower socioeconomic standing, improper disposal of human waste, and a lack of cleanliness in homes (Tshikuka *et al*., 1995; Holland *et al*., 1996; Gamboa *et al*., 1998, 2003).

In addition, because liver flukes can begin to form in the bile duct at a young age, possibly as early as 30-40 years, the risk factor of *O. viverrini* infection in young children is pertinent to the development of CCA. Patients who have had liver fluke infections throughout childhood may develop opisthorchiasis as a result of these infections (Sithithaworn & Haswell-Elkins, 2003; Sayasone *et al*., 2007; Andrews *et al*., 2008). How the liver flukes induce are associated with CCA? When the hepatobiliary cell stimulated by *O. viverrini* soluble ES products from liver flukes, human biliary cell lines proliferated excessively (Sripa, 2003). They have been discovered a human granulin homologue in the ES of *O. viverrini*, a powerful growth factor involved in cell proliferation and wound repair. The *O. viverrini* granulin, also known as Ov-GRN-1, was found in the biliary epithelial cells of experimentally infected hamsters in addition to being expressed in the majority of parasite organs, including the gut and tegument. Murine fibroblasts' proliferation was enhanced by recombinant Ov-GRN-1, and the afflicted cells underwent morphological alterations as a result. Antibody against Ov-GRN-1 inhibited Human CCA cell lines proliferated when exposed to *O. viverrini* ES, suggesting that Ov-GRN-1 is an important growth factor. According to their findings, liver fluke granulin is important in creating a tumor-genic environment infected livers that may eventually result in CCA (Smout *et al*., 2009). Nevertheless, certain regions of countrywide have been found to have an *O. viverrini* infection prevalence in the general population of above 20%, however data on the morbidity and mortality associated with liver fluke infection are still ambiguous (Khieu *et al*., 2019). In general speaking, further research on liver fluke infection is required to assess the risk factors for *O. viverrini* infection that are connected to an increase in the incidence of opisthorchiasis that associated to CCA development (Keiser & Utzinger, 2009; Sripa *et al*., 2010). On the other hand, *O. viverrini* infection is linked to CCA and was described as a family issue that affects mostly men since they are the primary breadwinners in their families (Andrews *et al*., 2008).

The epidemiology of liver fluke infection varies by endemic area, where foodborne trematode infection is widespread, especially in connection to the attitude of consumer culture and lifestyle. One of the reasons may be that the major source of infection in the surveyed areas is traditional fish dishes popularly consumed by the people regardless of gender, such as '*plea tre*' (fish salad), '*plea tre chou*' (sour fish salad), and '*ma'am*' (fermented fish, kept for 2–5 days) (Jung *et al*., 2023). The classification of risk factors for liver fluke infection based on parasitological and molecular testing is another frequent claim made in studies of demographics. Evidently, northeast Thailand has the highest prevalence (70%) of *O. viverrini* (Sripa & Pairojkul, 2008). In the southern Saravane district of Lao PDR, 58.5% of people had *O. viverrini* infection, which was the greatest incidence (Sayasone *et al*., 2007). However, prevalence rates in Vietnam's southern regions have been reported to range from 15.2% to 36.9% (De *et al*., 2003). The very recently publication was reported the emerging of *O. vierrini* infection in Preah Vihear

and Stung Treng Province. There appear to be certain elements that might make the investigated locations turn out to be opisthorchiasis hotspots. One is the vicinity of the communities that were surveyed. These provinces are found along the Mekong River's main stem or major tributaries (Jung *et al*., 2023). The 5 villages that make up Preah Vihear province are located right at the Cambodia-Lao PDR border. *O. viverrini* infection has been reported to be very prevalent in Lao PDR, particularly in the Vientiane Municipality, Khammouane, Savannakhet, and Champasak Province (Chai *et al*., 1998, 2005, 2007, 2009; Kobayashi *et al*., 2000). The five villages of Stung Treng province are close to the border and are situated along rivers and streams. The majority of villagers work in the fishing industry and have historically consumed freshwater fish uncooked or undercooked. These are additional risk factors (Jung *et al*., 2023). The location with the highest prevalence (9.94%) was Trapaing Srae village, Snuol district, Kratie Province, is bordering to Stung Treng Province but unremarkable at high-risk factor determined in this study. In summary, the demographic risk variables associated with the importance of the covariates age group, education level, employment, location were the main focus of the study at endemic areas. Such a sign can be used to inform the public and the authorities about untreated foodborne trematode infections, in an effort to increase awareness of opisthorchiasis and take action to control *O. viverrini* infection (Sripa & Pairojkul, 2008; Zhou *et al*., 2009). School-aged children have the greatest burden of illness brought on by intestinal nematodes when compared to other age groups. According to a mathematical model, treating schoolchildren alone can reduce the burden of disease on the entire population in high incidence neighborhoods by almost 70% (Chan, 1997).

In conclusion, this study sheds fresh light on *O. viverrini* infection and identifies age group as a determinant to predict prevalence. As consumers of secondary intermediate host species, humans' behavior can be influenced by controlling and reducing *O. viverrini* infection in the Snuol district, Kratie Province, Cambodia. The primary element that contributes to a high risk of transmission in the community is food consumption habit. The sociocultural elements that influence intestinal helminth transmission based in several research, parasitosis are correlated with illiteracy, a lack of latrines, diarrhea, a lower socioeconomic standing, improper disposal of human waste, and a lack of cleanliness in homes. The prevalence of *O. viverrini* infection in Kratie Province has to be addressed, and community-based health interventions that promote the consumption of freshwater fish that has been prepared properly should be put into place.

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Conflict of interest

The authors declare no competing interests.

REFERENCES

Andrews, R.H., Sithithaworn, P. & Petney, T.N. (2008). *Opisthorchis viverrini*: an underestimated parasite in world health. *Trends in Parasitology* **24**: 497-501. https://doi.org/10.1016/j.pt.2008.08.011

Aung, W.P.P., Htoon, T.T., Tin, H.H., Thinn, K.K., Sanpool, O., Jongthawin, J., Sadaow, L., Phosuk, I., Rodpai, R., Intapan, P.M. *et al*. (2017). First report and molecular identification of *Opisthorchis viverrini* infection in human communities from lower Myanmar. *PloS One* **12**: e0177130. https://doi.org/10.1371/journal.pone.0177130

Bundy D.A. (1988). Gender-dependent patterns of infections and disease. *Parasitology Today* **4**: 186-189. https://doi.org/10.1016/0169-4758(88)90076-2

Conlan, J.V., Sripa, B., Attwood, S. & Newton, P.N. (2011). A review of parasitic zoonoses in a changing Southeast Asia. *Veterinary Parasitology* **182**: 22-40. https://doi.org/10.1016/j.vetpar.2011.07.013

Chai, J.Y. & Jung, B.K. (2022). General overview of the current status of human foodborne trematodiasis. *Parasitology* **149**: 1262-1285. https://doi.org/10.1017/S0031182022000725

Chai, J.Y. & Hongvanthong, B. (1998). A small-scale survey of intestinal helminthic infections among the residents near Pakse, Laos. *The Korean Journal of Parasitology* **36**: 55-58. https://doi.org/10.3347/kjp.1998.36.1.55

Chai, J.Y., Park, J.H., Han, E.T., Guk, S.M., Shin, E.H., Lin, A., Kim, J.L., Sohn, W.M., Yong, T.S., Eom, K.S. *et al*. (2005). Mixed infections with *Opisthorchis viv*errini and intestinal flukes in residents of Vientiane Municipality and Saravane Province in Laos. *Journal of Helminthology* **79**: 283-289. https://doi.org/10.1079/joh2005302

Chai, J.Y., Han, E.T., Guk, S.M., Shin, E.H., Sohn, W.M., Yong, T.S., Eom, K.S., Lee, K.H., Jeong, H.G., Ryang, Y.S. *et al*. (2007). High prevalence of liver and intestinal fluke infections among residents of Savannakhet Province in Laos. *The Korean Journal of Parasitology* **45**: 213-218. https://doi.org/10.3347/kjp.2007.45.3.213

Chai, J.Y., Han, E.T., Shin, E.H., Sohn, W.M., Yong, T.S., Eom, K.S., Min, D.Y., Um, J.Y., Park, M.S., Hoang, E.H. *et al*. (2009). High prevalence of *Haplorchis taichui*, *Phaneropsolus molenkampi*, and other helminth infections among people in Khammouane province, Lao PDR. *The Korean Journal of Parasitology* **47**: 243-247. https://doi.org/10.3347/kjp.2009.47.3.243

Chan, M.S. (1997). The global burden of intestinal nematode infections – fifty years on. *Parasitology Today* **13**: 438-443. https://doi.org/10.1016/s0169-4758(97)01144-7

De, N.V., Murrell, K.D., Cong, leD., Cam, P.D., Chau, leV., Toan, N.D. & Dalsgaard, A. (2003). The food-borne trematode zoonoses of Vietnam. *The Southeast Asian Journal of Tropical Medicine and Public Health* **34**: 12-34.

Duenngai, K., Sithithaworn, P., Rudrappa, U.K., Iddya, K., Laha, T., Stensvold, C.R., Strandgaard, H. & Johansen, M.V. (2008). Improvement of PCR for detection of *Opisthorchis viverrini* DNA in human stool samples. *Journal of Clinical Microbiology* **46**: 366-368. https://doi.org/10.1128/JCM.01323-07

Forrer, A. (2012). Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in Southern Lao PDR. *PLoS Neglected Tropical Diseases* **6**: 1481. https://doi.org/10.1371/journal.pntd.0001481

Fried, B. & Abruzzi, A. (2010). Food-borne trematode infections of humans in the United States of America. *Parasitology Research* **106**: 1263-1280. https://doi.org/10.1007/s00436-010-1807-0

Gamboa, M.I., Basualdo, J.A., Kozubsky, L., Costas, E., Cueto Rua, E. & Lahitte, H.B. (1998). Prevalence of intestinal parasitosis within three population groups in La Plata, Argentina. *European Journal of Epidemiology* **14**: 55-61. https://doi.org/10.1023/a:1007479815249

Gamboa, M.I., Basualdo, J.A., Córdoba, M.A., Pezzani, B.C., Minvielle, M.C. & Lahitte, H.B. (2003). Distribution of intestinal parasitoses in relation to environmental and sociocultural parameters in La Plata, Argentina. *Journal of Helminthology* **77**: 15-20. https://doi.org/10.1079/JOH2002142

Holland, C.V., Asaolu, S.O., Crompton, D.W., Whitehead, R.R. & Coombs, I. (1996). Targeted anthelminthic treatment of school children: effect of frequency of application on the intensity of *Ascaris lumbricoides* infection in children from rural Nigerian villages. *Parasitology* **113**: 87-95. https://doi.org/10.1017/s0031182000066300

Hudson, P.J. & Dobson, A.P. (1995). Macroparasites: observed patterns. In: Ecology of Infectious Diseases in Natural Populations, Grenfell, B.T & Dobson, A.P. (editors). Cambridge: Cambridge University Press, pp. 144-176.

Jung, B.K., Hong, S., Chang, T., Cho, J., Ryoo, S., Lee, K.H., Lee, J., Sohn, W.M., Hong, S.J., Khieu, V. *et al*. (2023). High endemicity of *Opisthorchis viverrini* infection among people in northern Cambodia confirmed by adult worm expulsion. *Scientific Reports* **13**: 9654. https://doi.org/10.1038/s41598-023-36544-z

Kaewkes, S. (2003). Taxonomy and biology of liver flukes. *Acta Tropica* **88**: 177-186. https://doi.org/10.1016/j.actatropica.2003.05.001

Kaewpitoon, N., Kaewpitoon, S.J. & Pengsaa, P. (2008). Opisthorchiasis in Thailand: review and current status. *World Journal of Gastroenterology* **14**: 2297-2302. https://doi.org/10.3748/wjg.14.2297

Keiser, J. & Utzinger, J. (2005). Emerging foodborne trematodiasis. *Emerging Infectious Diseases* **11**: 1507-1514. https://doi.org/10.3201/eid1110.050614

Keiser, J. & Utzinger, J. (2009). Food-borne trematodiases. *Clinical Microbiology Reviews* **22**: 466-483. https://doi.org/10.1128/CMR.00012-09

Khieu, V., Fürst, T., Miyamoto, K., Yong, T.S., Chai, J.Y., Huy, R., Muth, S. & Odermatt, P. (2019). Is *Opisthorchis viverrini* emerging in Cambodia?. *Advances in Parasitology* **103**: 31-73. https://doi.org/10.1016/bs.apar.2019.02.002

Kobayashi, J., Vannachone, B., Sato, Y., Manivong, K., Nambanya, S. & Inthakone, S. (2000). An epidemiological study on *Opisthorchis viverrini* infection in Lao villages. *The Southeast Asian Journal of Tropical Medicine and Public Health* **31**: 128-132.

La, N., Leng, M., Rattanapitoon, K., Pechdee, P., Boonsuya, A., Arunsan, P. & Rattanapitoon, S.K. (2022). Intestinal parasitic infections and risk factors among the population in Cambodia. *Tropical Biomedicine* **39**: 539-546. https://doi.org/10.47665/tb.39.4.009

Lee, K.J., Bae, Y.T., Kim, D.H., Deung, Y.K., Ryang, Y.S., Kim, H.J., Im, K.I. & Yong, T.S. (2002). Status of intestinal parasites infection among primary school children in Kampongcham, Cambodia. *The Korean Journal of Parasitology* **40**: 153-155. https://doi.org/10.3347/kjp.2002.40.3.153

National Institute of Statistic (NIS), Ministry of Planning. (2020). General populations census of the Kingdom of Cambodia 2019. In: National report, National Institute of Statistic, Ministry of Planning. Cambodia: Ministry of Planning, pp 16.

Miyamoto, K., Kirinoki, M., Matsuda, H., Hayashi, N., Chigusa, Y., Sinuon, M., Chuor, C.M. & Kitikoon, V. (2014). Field survey focused on *Opisthorchis viverrini* infection in five provinces of Cambodia. *Parasitology International* **63**: 366-373. https://doi.org/10.1016/j.parint.2013.12.003

Provincial map. (2010). Map of Cambodia highlighting Krati'. Wikipedia. orgWeb. https://en.wikipedia.org/wiki/Krati%C3%A9_province#/media/File:Cambodia_Krati%C3%A9_locator_map.svg. Assessed 02 February 2010.

Nematian, J., Nematian, E., Gholamrezanezhad, A. & Asgari, A.A. (2004). Prevalence of intestinal parasitic infections and their relation with socio-economic factors and hygienic habits in Tehran primary school students. *Acta Tropica* **92**: 179-186. https://doi.org/10.1016/j.actatropica.2004.06.010

Ngrenngarmlert, W., Kritsiriwuthinan, K. & Nilmanee, N. (2012). Prevalence of intestinal parasitic infections among Myanmar workers in Bangkok and Samut Sakhon. *Asia Journal of Public Health* **3**: 53-58.

Radomyos, B., Wongsaroj, T., Wilairatana, P., Radomyos, P., Praevanich, R., Meesomboon, V. & Jongsuksuntikul, P. (1998). Opisthorchiasis and intestinal fluke infections in northern Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health* **29**: 123-127.

Pengput, A. & Schwartz, D.G. (2020). Risk factors for *Opisthorchis viverrini* infection: a systematic review. *Journal of Infection and Public Health* **13**: 1265-1273. https://doi.org/10.1016/j.jiph.2020.05.028

Rim, H.J., Chai, J.Y., Min, D.Y., Cho, S.Y., Eom, K.S., Hong, S.J., Sohn, W.M., Yong, T.S., Deodato, G., Standgaard, H. *et al*. (2003, Oct). Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. *Parasitology Research* **91**: 267-272. https://doi.org/10.1007/s00436-003-0963-x

Rim, H.J. (1990). Clonorchiasis in Korea. *Kisaengchunghak Chapchi* **28**: 63-78. https://doi.org/10.3347/kjp.1990.28.suppl.63

Sayasone, S., Odermatt, P., Phoumindr, N., Vongsaravane, X., Sensombath, V., Phetsouvanh, R., Choulamany, X. & Strobel, M. (2007). Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**: 40-47. https://doi.org/10.1016/j.trstmh.2006.02.018

Sermswan, R., Mongkolsuk, S., Panyim, S. & Sirisinha, S. (1991). Isolation and characterization of *Opisthorchis viverrini* specific DNA probe. *Molecular and Cellular Probes* **5**: 399-407. https://doi.org/10.1016/s0890-8508(05)80011-8

Sithithaworn, P. & Haswell-Elkins, M. (2003). Epidemiology of *Opisthorchis viverrini*. *Acta Tropica* **88**: 187-194. https://doi.org/10.1016/j.actatropica.2003.02.001

Sithithaworn, P., Nuchjungreed, C., Srisawangwong, T., Ando, K., Petney, T.N., Chilton, N.B. & Andrews, R.H. (2007). Genetic variation in *Opisthorchis viverrini* (Trematoda: Opisthorchiidae) from northeast Thailand and Laos PDR based on random amplified polymorphic DNA analyses. *Parasitology Research* **100**: 613-617. https://doi.org/10.1007/s00436-006-0304-y

Smout, M.J., Laha, T., Mulvenna, J., Sripa, B., Suttiprapa, S., Jones, A., Brindley, P.J. & Loukas, A. (2009). A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, promotes proliferation of host cells. *PLoS Pathogens* **5**: 1000611. https://doi.org/10.1371/journal.ppat.1000611

Sohn, W.M., Yong, T.S., Eom, K.S., Pyo, K.H., Lee, M.Y., Lim, H., Choe, S., Jeong, H.G., Sinuon, M., Socheat, D. *et al*. (2012). Prevalence of *Opisthorchis viverrini* infection in humans and fish in Kratie province, Cambodia. *Acta Tropica* **124**: 215-220. https://doi.org/10.1016/j.actatropica.2012.08.011

Sripa, B., Kaewkes, S., Intapan, P.M., Maleewong, W. & Brindley, P.J. (2010). Food-borne trematodiases in Southeast Asia epidemiology, pathology, clinical manifestation and control. *Advances in Parasitology* **72**: 305-350. https://doi.org/10.1016/S0065-308X(10)72011-X

Sripa, B. & Pairojkul, C. (2008). Cholangiocarcinoma: lessons from Thailand. *Current Opinion in Gastroenterology* **24**: 349-356. https://doi.org/10.1097/MOG.0b013e3282fbf9b3

Sripa, B., Suwannatrai, A.T., Sayasone, S., Do, D.T., Khieu, V. & Yang, Y. (2021). Current status of human liver fluke infections in the Greater Mekong Subregion. *Acta Tropica* **224**: 106133. https://doi.org/10.1016/j.actatropica.2021.106133

Sripa, B. (2003). Pathobiology of opisthorchiasis: an update. *Acta Tropica* **88**: 209-220. https://doi.org/10.1016/j.actatropica.2003.08.002

Suwannahitatorn, P., Webster, J., Riley, S., Mungthin, M. & Donnelly, C.A. (2019). Uncooked fish consumption among those at risk of *Opisthorchis viverrini* infection in central Thailand. *PloS One* **14**: 0211540. https://doi.org/10.1371/journal.pone.0211540

Thaenkham, U., Nuamtanong, S., Vonghachack, Y., Yoonuan, T., Sanguankiat, S., Dekumyoy, P., Prommasack, B., Kobayashi, J. & Waikagul, J. (2011). Discovery of *Opisthorchis lobatus* (Trematoda: Opisthorchiidae): a new record of small liver flukes in the Greater Mekong Sub-region. *The Journal of Parasitology* **97**: 1152-1158. https://doi.org/10.1645/GE-2764.1

Thaenkham, U., Nuamtanong, S., Sa-nguankiat, S., Yoonuan, T., Touch, S., Manivong, K., Vonghachack, Y., Sato, M. & Waikagul, J. (2010). Monophyly of *Opisthorchis viverrini* populations in the lower Mekong Basin, using mitochondrial DNA nad1 gene as the marker. *Parasitology International* **59**: 242-247. https://doi.org/10.1016/j.parint.2010.02.009

Thu, N.D., Dalsgaard, A., Loan, L.T. & Murrell, K.D. (2007). Survey for zoonotic liver and intestinal trematode metacercariae in cultured and wild fish in An Giang province, Vietnam. *The Korean Journal of Parasitology* **45**: 45-54. https://doi.org/10.3347/kjp.2007.45.1.45

Traub, R.J., Macaranas, J., Mungthin, M., Leelayoova, S., Cribb, T., Murrell, K.D. & Thompson, R.C. (2009). A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to central Thailand. *PLoS Neglected Tropical Diseases* **3**: 367. https://doi.org/10.1371/journal.pntd.0000367

Tshikuka, J.G., Scott, M.E. & Gray-Donald, K. (1995). *Ascaris lumbricoides* infection and environmental risk factors in an urban African setting. *Annals of Tropical Medicine and Parasitology* **89**: 505-514. https://doi.org/10.1080/00034983.1995.11812983

WHO (World Health Organization). (2020). Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030. Geneva, Switzerland: WHO (2020). http://www.who.int/publications/i/item/9789240010352 Accessed 28 January 2021.

Wattanawong, O., Iamsirithaworn, S., Kophachon, T., Nak-Ai, W., Wisetmora, A., Wongsaroj, T., Dekumyoy, P., Nithikathkul, C., Suwannatrai, A.T. & Sripa, B. (2021). Current status of helminthiases in Thailand: A cross-sectional, nationwide survey, 2019. *Acta Tropica* **223**: 106082. https://doi.org/10.1016/j.actatropica.2021.106082

Wongratanacheewin, S., Pumidonming, W., Sermswan, R.W., Pipitgool, V. & Maleewong, W. (2002). Detection of *Opisthorchis viverrini* in human stool specimens by PCR. *Journal of Clinical Microbiology* **40**: 3879-3880. https://doi.org/10.1128/JCM.40.10.3879-3880.2002

Yong, T.S., Shin, E.H., Chai, J.Y., Sohn, W.M., Eom, K.S., Lee, D.M., Park, K., Jeoung, H.G., Hoang, E.H., Lee, Y.H. *et al*. (2012). High prevalence of *Opisthorchis viverrini* infection in a riparian population in Takeo province, Cambodia. *The Korean Journal of Parasitology* **50**: 173-176. https://doi.org/10.3347/kjp.2012.50.2.173

Yong, T.S., Chai, J.Y., Sohn, W.M., Eom, K.S., Jeoung, H.G., Hoang, E.H., Yoon, C.H., Jung, B.K., Lee, S.H., Sinuon, M. *et al*. (2014). Prevalence of intestinal helminths among inhabitants of Cambodia (2006-2011). *The Korean Journal of Parasitology* **52**: 661-666. https://doi.org/10.3347/kjp.2014.52.6.661

Zhao, T.T., Feng, Y.J., Doanh, P.N., Sayasone, S., Khieu, V., Nithikathkul, C., Qian, M.B., Hao, Y.T. & Lai, Y.S. (2021). Model-based spatial-temporal mapping of opisthorchiasis in endemic countries of Southeast Asia. *eLife* **10**: e59755. https://doi.org/10.7554/eLife.59755

Zhou, X.N., Lv, S., Yang, G.J., Kristensen, T.K., Bergquist, N.R., Utzinger, J. & Malone, J.B. (2009). Spatial epidemiology in zoonotic parasitic diseases: insights gained at the 1st International Symposium on Geospatial Health in Lijiang, China, 2007. *Parasites & Vectors* **2**: 10. https://doi.org/10.1186/1756-3305-2-10

Zuk, M. (1990). Reproductive strategies and disease susceptibility: an evolutionary viewpoint. *Parasitology Today* **6**: 231-233. https://doi.org/10.1016/0169-4758(90)90202-f

Zuk, M. & McKean, K.A. (1996). Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology* **26**: 1009-1023. https://doi.org/10.1016/S0020-7519(96)80001-4