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

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ARTICLE HISTORY

Received: 30 June 2023
Revised: 23 August 2023
Accepted: 27 August 2023
Published: 31 December 2023

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 Tra paing 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 viverrini*; 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 trematodiasis, 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...
The prevalence of liver flukes infection to human was reported over 600 million people at high risk of infection with this neglected trematode (Keiser & Uzting, 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 overseas 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 geographical boundary of Kratie Province bordering to Stung Treng, Kampong Thom, Kampong Cham, Tboung Khmorn, Mondulkiri Province, and South Vietnam. The Greater Mekong River Subregion extending flow a cross 6 province in Cambodia including Stung Treng, Kratie, Tboung Khmorn, 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 (Kawekes, 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: \( n = \frac{N(2a/2)(1-P)/\text{e}^2}{N-1} \), which is represented by \( n \) the sample size or population, \( P \) prevalence or proportion of infected individuals, \( a = 2/2 = \text{normal deviation for two-tailed alternative hypothesis at a level of significance of 1.96, and e \text{precision margin of error (5% or 0.05}} \) (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 Traiping Srae, Chreb, Cheung Khie, Pravanh, and Cheung Khlu villages were in the Pithnu subdistrict, Snoul 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: NRP013) 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, Snoul 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'-3'-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 H2O, 2.5 µL of 10x Taq buffer, 1 µL of dNTP, 1 µL of ITS2-OvF, 1 µL of ITS2-OvR, 0.2 µL of Taq DNA polymerase, 1.5 µL of Mgc2+, 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 (Duennngai et al., 2008). The
PCR products were amplified using the G-STORM™ 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: A5584735), 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
<th><em>O. viverrini</em> n (%)</th>
<th>OR OR (95% CI)</th>
<th>P-value</th>
<th>OR adj (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Male</td>
<td>157 (41.64)</td>
<td>14 (8.92)</td>
<td>0.589 (0.265–1.311)</td>
<td>0.195</td>
<td>0.626 (0.269–1.457)</td>
<td>0.277</td>
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<tr>
<td>Female</td>
<td>220 (58.36)</td>
<td>12 (5.45)</td>
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<tr>
<td>Age groups</td>
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<tr>
<td>&lt; 20 years</td>
<td>97 (25.73)</td>
<td>13 (13.40)</td>
<td>0.569 (0.388–0.836)</td>
<td>0.004*</td>
<td>0.601 (0.410–0.882)</td>
<td>0.009*</td>
</tr>
<tr>
<td>21-30 years</td>
<td>104 (27.59)</td>
<td>8 (7.69)</td>
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<td>31-40 years</td>
<td>70 (18.57)</td>
<td>2 (2.86)</td>
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<td>41-50 years</td>
<td>58 (15.38)</td>
<td>2 (3.45)</td>
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<tr>
<td>&gt;51 years</td>
<td>48 (12.73)</td>
<td>1 (2.08)</td>
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<tr>
<td>Education level</td>
<td></td>
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<tr>
<td>Illiteracy</td>
<td>103 (27.32)</td>
<td>9 (8.74)</td>
<td>0.696 (0.422–1.148)</td>
<td>0.156</td>
<td>0.765 (0.451–1.299)</td>
<td>0.322</td>
</tr>
<tr>
<td>Primary</td>
<td>133 (35.28)</td>
<td>11 (8.27)</td>
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<td>Secondary and higher</td>
<td>141 (37.40)</td>
<td>6 (4.26)</td>
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<tr>
<td>Occupation</td>
<td></td>
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<tr>
<td>Employee</td>
<td>41 (10.88)</td>
<td>2 (4.88)</td>
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<tr>
<td>Farmer</td>
<td>144 (38.20)</td>
<td>12 (8.33)</td>
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<tr>
<td>Shopkeeper</td>
<td>68 (18.04)</td>
<td>4 (5.88)</td>
<td>1.052 (0.810–1.368)</td>
<td>0.703</td>
<td>1.022 (0.776–1.347)</td>
<td>0.875</td>
</tr>
<tr>
<td>Housewife</td>
<td>62 (16.45)</td>
<td>2 (3.23)</td>
<td></td>
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<tr>
<td>Government - officer</td>
<td>19 (5.04)</td>
<td>1 (5.26)</td>
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<tr>
<td>Others</td>
<td>43 (11.41)</td>
<td>5 (11.63)</td>
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<tr>
<td>Location</td>
<td></td>
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<tr>
<td>Trapeang Srae</td>
<td>161 (42.71)</td>
<td>16 (9.94)</td>
<td>0.900 (0.681–1.189)</td>
<td>0.460</td>
<td>0.934 (0.701–1.243)</td>
<td>0.638</td>
</tr>
<tr>
<td>Chrab</td>
<td>63 (16.71)</td>
<td>1 (1.59)</td>
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<tr>
<td>Cheung Khie</td>
<td>52 (13.79)</td>
<td>1 (1.92)</td>
<td></td>
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<tr>
<td>Pravan</td>
<td>42 (11.14)</td>
<td>4 (9.52)</td>
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<tr>
<td>Cheung Khue</td>
<td>59 (15.65)</td>
<td>4 (6.78)</td>
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*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 adj)=0.569, 95% CI=0.388-0.836, P=0.004 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 years (n=8, 7.69%), 31-40 years (n=2, 2.86%), 41-50 years (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 Trapeang 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 (*nad1*) among the population in Kratie Province (Sohn et al., 2012). In the molecular marker including the ITS2, cytochrome c oxidase subunit 1 (cox1), and *nad1* 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, *nad1*-based PCR was utilized to reveal homologous nucleotide sequences (97-99%). ITS2 sequenced was more specificity than cytochrome c oxidase subunit 1 (cox1), with 0.86% and 3.03% nucleotide differences between *O. viverrini* and *Opisthorchis lobatis*, 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%) Ratankiri 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 Snouol 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 Snouol 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 (Sitthithaworn & Haswell-Elkins, 2003; Kaewpitoon et al., 2009). 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., 2019). 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 (Sitthithaworn & 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).

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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, Snoul 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 Snoul 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 helmminh 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.

ACKNOWLEDGEMENTS

This study was supported by Suranaree University of Technology’s grant “SUT Scholarship for Graduate International Students (Vithedhunbti)” dated 7 October 2016. Suranaree University of Technology (SUT), Thailand Science Research and Innovation (TSRI), NRIS number 179304, National Science, Research, and Innovation Fund (NSRF). The authors would like to acknowledge the Parasitic Diseases Research Center (PDCR), Institute of Medicine and BioEthic Committee, Institute of Research and Development. The Human Ethics Committee of the Health office of Nakhon Ratchasima Province and Ministry of Health of Cambodia. At last, greatly appreciate to all subjects who participated in this research.

Conflict of interest

The authors declare no competing interests.

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