RESEARCH ARTICLE

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# Re- Infection Rate of Opisthorchis Viverrini Five Years After Treatment with Praziquantel in High-Risk Area: A Community-Based Study

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## Abstract

**Background:** *Opisthorchis viverrini* (OV) is a significant public health problem in Thailand, and OV reinfection poses a serious risk of cholangiocarcinoma with little evidence of a decrease. While numerous studies have explored OV reinfection and prevalence, most have been limited to short study period. Therefore, there is a need for long-term investigations to gather sufficient evidence. **Objectives:** This study aimed to access the current status of re-infection rates in high- risk areas and to determine associations between factors of and re-infection of OV at 5 years after treatment with praziquantel. **Materials and Methods:** In total, this study included 457 participants from a cohort study. Each participant was required to answer a questionnaire and undergo stool examination using the formalin ethyl acetate concentration technique. Data were analyzed using descriptive statistics and multiple logistic regression. **Result:** Out of the total 457 participants, 418 (91.5%) successfully completed the questionnaires and underwent stool examinations. Among the participants, 10.8% showed re-infection with OV. Using multivariate analysis, factors significantly associated with reinfection OV included yearly income (OR adj = 2.14, 95%CI = 1.11- 4.12, p-value = 0.022) and past stool examination five years (OR adj = 2.47, 95%CI = 1.13-5.43, p-value = 0.023), respectively. **Conclusions:** Subjects who frequently experience OV reinfection should undergo CCA screening by experts while closely monitoring their raw fish consumption behavior. Moreover, it is essential to implement comprehensive programs aimed at promoting behavioral changes and provide preventive education, with a specific focus on high epidemic areas, to discourage the consumption of raw fish.

**Keywords:** re-infection rate- *Opisthorchis viverrini*- praziquantels- Thailand

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## Introduction

Re-infection with *Opisthorchis viverrini* (OV) and chronic OV infection pose significant concerns as they are major risk factors for cholangiocarcinoma (CCA), presenting a crucial public health challenge in Thailand. In countries where OV is endemic, intrahepatic CCA accounts for 40% and extrahepatic CCA for 60% of all cases [1]. The prevalence of CCA is notably high, particularly in the northeast region, and prospective studies have reported a cumulative re-infection rate of 10.9% after one year of praziquantel treatment [2]. Re-infection rates have been observed to be higher among males, especially those aged over 50 years, individuals with completed primary education, and those engaged in agricultural occupation [2].

Raw fish consumption was identified as the primary risk factor for OV infection. The Thai Ministry of Public Health has continuously implemented a national program to reduce OV infection using the following three strategies for more than three decades: (1) conducting campaigns to encourage the cessation of raw fish consumption; (2) Providing health education to villagers and students, aiming to promote positive health behaviors. (3) stool examinations of risk groups and treatment of infected cases with praziquantel [3]. Since 2015, a new recommendation called Eco health has been adopted to control OV [4], the national Opisthorchis's control program encountered reduced government funding and diverted resources to other priorities [1]. Consequently, a national program (2014-2018) was re-started to eradicate OV infection and reduce CCA cases [5, 6], in collaboration with Khon Kaen University and the Ministry of Public Health to promote public health policy overall.

However, numerous studies have consistently reported that the prevalence of OV remains high in all regions of Northeast Thailand. For instance, in Yasothon Province, the prevalence of OV infection was found to

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be 38.7% [7], while in Loie Province, it was 15.6% [8]. A recent study investigating risk areas in Health Region 7, comprising Roi-et, Maha Sarakham, Khon Kaen, and Kalasin Provinces, reported a prevalence of 14.9% for OV [9]. These findings suggest that while the prevalence of OV infection appeared to have marginally decrease overall, it remains notably high in wetlands regions. In terms of current treatment and control of liver fluke infection, praziquantel also has been widely shown to have high efficacy for treating OV infection; however, it has a relatively short half-life and does not protect from the infection long term [10]. Although the parasite is eliminated by praziquantel, the pathology of OV infection ensures progression to CCA [11]. Furthermore, OV infection can occur early in life, particularly among high-risk individuals, especially those who have been previously infected, and can recur at any time during the transmission cycle. Therefore, the high prevalence of OV and reinfection rate after praziquantel treatment are common patients who continue to consume raw fish. The majority of the aforementioned studies utilized cross-sectional designs, which assessed the prevalence of OV over a short period, typically less than one year. However, there is a limited number of studies that have provide comprehensive data on OV reinfections and evaluated the long-term effectiveness of health education interventions in preventing such reinfection. As a result, there is a need for more research to gather long-term evidence regarding re-infection rate with OV and to assess the impact of improved health education strategies.

This paper focuses on surveillance of the re-infection rate at 5 years and identifies factors associated with OV re-infection after treatment with praziquantel in older subjects who were previously cured and have been residing in high-risk areas since then. The finding of this study hold significant potential to support the national program's efforts in implementing effective control measures in rural areas for the third decade in the northeast region. Furthermore, the study results can contribute to sustaining concern and raising awareness about preventing liver fluke disease among villagers.

## Materials and Methods

This survey was conducted between September to November 2017. The population and sample size were based on a related prospective study [7] that enrolled 457 subjects from Hua Mueang, a subdistrict of Maha Chana Chai, and Kut Kung, a subdistrict of Kham Khuean Kaew. These subdistricts have experienced high OV infection rates in recent years, particularly in 2012, owing to their geographical characteristics, with numerous swamps and ponds in close proximity to the Chi River in the southern part of Yasothon Province, northeast Thailand. The two subdistricts were selected using two multistage sampling techniques from the nine provincial districts [7]. All subjects aged 25 to 75 years who were still alive and residing in either of the study areas for at least six months were invited to participate in the study and interested volunteers provided stool samples analyzed using the formalin ethyl acetate concentration technique [12] by laboratory staff within 24 h of collection. Individuals, who reported taking praziquantel in the week before stool collection (n=3), who died (n = 4), who were pregnant (n=2), or who were unable to provide data such as those with severe illness (n = 3), were excluded from the study (As shown in Figure 1). A structured questionnaire was used to collect information about sociodemographic, eating uncooked fish, and other related factors, the data were summarized using descriptive statistics, and the chi-square test was used to analyze the associations between variables and re-infection with OV. The multivariate analysis comprised multiple logistic regression with the backward elimination of variables to adjust for confounding factors. The statistical significance of the final model was set at $p<0.05$.

## Results

A total of 418 subjects (91.46%) included in the study completed the interview questionnaire and provided the necessary stool samples. Among these participants, 45 subjects (10.8%) were found to have been reinfected with OV five years after being treated with praziquantel.

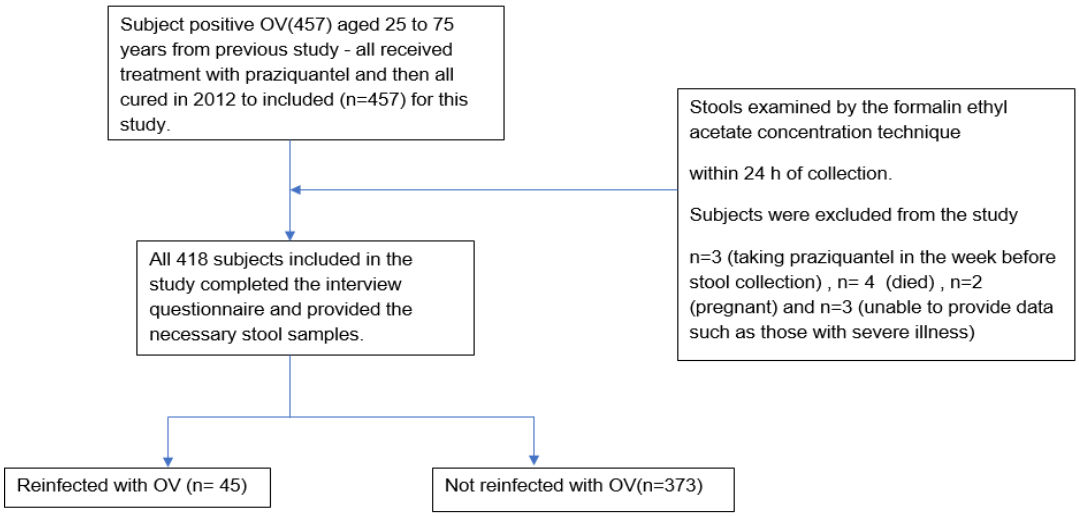


Figure 1. The Process of Selecting Subjects was Included for This Study and Final Analyses in Figure

Table 1. Characteristics of Participants

| Characteristics (n = 418) | OV reinfection | | | | Total | % |
|---|---|---|---|---|---|---|
| | Positive | % | Negative | % | | |
| Gender | | | | | | |
| Female | 22 | 10.3 | 192 | 89.7 | 214 | 51.2 |
| Male | 23 | 11.3 | 181 | 88.7 | 204 | 48.8 |
| Age, years | | | | | | |
| ≤40 | 4 | 13.3 | 26 | 86.7 | 30 | 7.2 |
| 41–60 | 30 | 12.4 | 211 | 87.6 | 241 | 57.7 |
| >60 | 11 | 7.5 | 136 | 92.5 | 147 | 35.2 |
| Education | | | | | | |
| Primary education | 31 | 9.3 | 302 | 90.7 | 333 | 79.7 |
| Secondary education or higher | 14 | 16.5 | 71 | 83.5 | 85 | 20.3 |
| Occupation | | | | | | |
| Other | 9 | 28.1 | 23 | 71.9 | 32 | 7.7 |
| Agriculture | 36 | 9.3 | 350 | 90.1 | 386 | 92.3 |
| Income (baht) | | | | | | |
| ≤30,000 | 25 | 8.6 | 265 | 91.4 | 290 | 69.4 |
| >30,000 | 20 | 15.6 | 108 | 84.4 | 128 | 30.6 |
| Consumption of raw fish in the past year | | | | | | |
| Yes | 32 | 10.7 | 267 | 89.3 | 299 | 71.5 |
| No | 13 | 10.9 | 106 | 89.1 | 119 | 28.5 |
| Stool examination | | | | | | |
| Yes | 29 | 8.9 | 298 | 91.1 | 327 | 78.2 |
| No | 16 | 17.6 | 75 | 82.4 | 91 | 21.8 |
| Knowledge of OV | | | | | | |
| Middle-Low | 22 | 10.8 | 182 | 89.2 | 204 | 48.8 |
| High | 23 | 10.7 | 191 | 89.3 | 214 | 51.2 |
| Treated with praziquantel in the past | | | | | | |
| Yes | 15 | 11.3 | 140 | 88.7 | 155 | 37.1 |
| No | 30 | 11.4 | 233 | 88.6 | 263 | 62.9 |

In Table 1, the overall, the re-infection rate of OV was nearly similar among males (11.3%) and females (10.3%). The majority of subjects belonged to the age groups of 25 to 40 years for females (18.7%) and 41 to 60 years for males (13.9). However, in the age group of 61 to 75 years, the re-infection rate of OV decreased for both sexes. Most of the subjects (16.5%) were educated and had completed secondary education or higher school, moreover; agriculture was the occupation of almost all subjects (10.9%). Most income (15.6%) had an income >

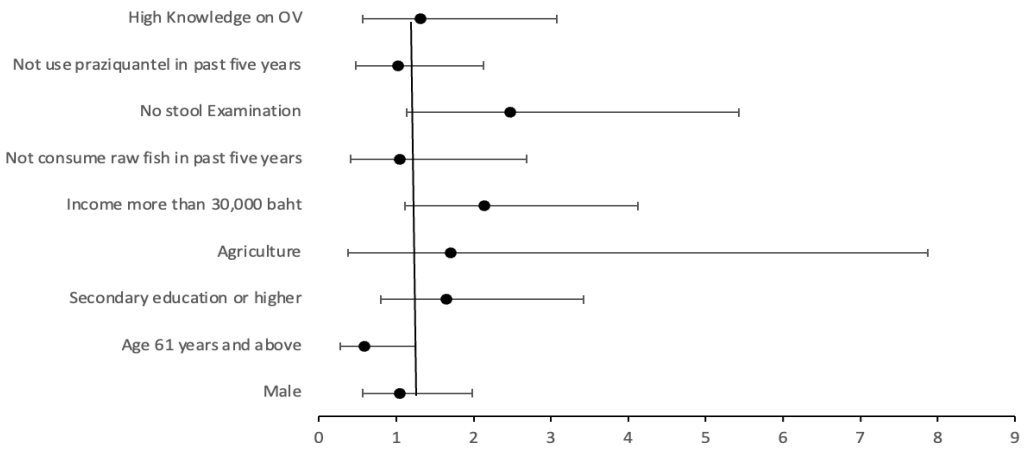


Figure 2. Forest Plot of Adjusted Odd Ratio for the Associated with *O.viverrini* Infection

Table 2. Factors associated with OV reinfection (n=418)

| Factors | OV reinfection | | Crude OR (95% CI) | P-value | Adjusted OR (95% CI) | P-value |
|---|---|---|---|---|---|---|
| | Positive | Negative | | | | |
| Gender | | | | | | |
| Female | 22 | 192 | 1 | | 1 | |
| Male | 23 | 181 | 1.11(0.59–2.05) | 0.743 | 1.05(0.56-1.98) | 0.877 |
| Age, years | | | | | | |
| ≤ 60 | 34 | 237 | 1 | | 1 | |
| > 60 | 11 | 136 | 0.56(0.27-1.14) | 0.115 | 0.59(0.27-1.25) | 0.173 |
| Education | | | | | | |
| Primary education | 31 | 302 | 1 | | 1 | |
| Secondary education or higher | 14 | 71 | 1.92(0.97–3.79) | 0.061 | 1.65(0.80–3.42) | 0.172 |
| Occupation | | | | | | |
| Other | 9 | 23 | 1 | | 1 | |
| Agriculture | 36 | 350 | 1.41(0.32–6.20) | 0.647 | 1.70(0.37-7.87) | 0.493 |
| Income (baht) | | | | | | |
| ≤30,000 | 25 | 265 | 1 | | 1 | |
| >30,000 | 20 | 108 | 1.96(1.04–3.68) | 0.036 | 2.14(1.11–4.12) | 0.022 |
| Consumption of raw fish in the past year | | | | | | |
| Yes | 32 | 267 | 1 | | 1 | |
| No | 13 | 106 | 1.02(0.52–2.03) | 0.947 | 1.05(0.41-2.68) | 0.911 |
| Stool examination | | | | | | |
| Yes | 29 | 298 | 1 | | 1 | |
| No | 16 | 75 | 2.22(1.13–4.24) | 0.02 | 2.47(1.13–5.43) | 0.023 |
| Knowledge of OV | | | | | | |
| Middle-Low | 22 | 182 | 1 | | 1 | |
| High | 23 | 191 | 0.99(0.53–1.83) | 0.99 | 1.31(0.56-3.08) | 0.524 |
| Treated with praziquantel in the past | | | | | | |
| Yes | 15 | 140 | 1 | | 1 | |
| No | 30 | 233 | 1.20(0.62-2.31) | 0.582 | 1.02(0.48-2.13) | 0.953 |

30,000 baht per year. The percentage of subjects who had and not consumed raw fish in the past year was similar (10.7% and 10.9%, respectively). There were more cases in which stool samples were not examined (17.6%). The percentages of subjects with middle-low and high knowledge of OV infection were approximately equal (10.8% and 10.7%, respectively).

As shown in Table 2 and Figure 2, the factors that were significantly associated with OV reinfection as analyzed using logistic regression were income (OR adj = 2.14, 95%CI = 1.11- 4.12, p-value = 0.022) and previous stool examination (OR adj = 2.47, 95%CI = 1.13-5.43, p-value = 0.023).

## Discussion

In the present study, the reported re-infection rate of OV five years after being treated with praziquantel was similar to that reported in a related cohort study [13] when the Thai Ministry of Public Health aimed to eradicate OV and CCA from 2014 to 2018 [5] by implementing a challenge strategy for the Thai population, particularly in northeast Thailand. The policy, driven by the National Health Assembly, included implementing measures and guidelines to screen CCA using ultrasound and stool examination in high-risk groups and among individuals in high-risk areas covering all northeastern provinces in Thailand [6]. Moreover, health education was provided to prevent re-infection with OV in high-risk groups. Knowledge and basic health regarding OV infection and its prevention were also provided to villagers and students, and they were advised to change their habit of consuming raw fish. As the main strategy in reducing the distribution and transmission cycle, villagers were advised to use the toilet, and the community was advised to participate in managing the environment and sewage discharge: thereby, preventing the formation of primary and secondary intermediate hosts and infection reservoirs of metacercaria. Therefore, activities to prevent and control were implemented for all the risk factors of OV infection. During the duration in which the policy was implemented, many studies reported the prevalence of OV infection in northeastern Thailand remained as high as 22.7% [14] and 1.74% [15]. A population-based cross-sectional study in northeast Thailand [16] found the current OV prevalence among participants having been treated with praziquantel

was 18.1%. The rates also reflected that the results of the strategy policy in this region failed to meet expectations. For a long time, the program still has made no impact in these areas and OV infection appears to be a neglected disease. According to the re-infection rate, ecological factors for example; social culture, economy, lifestyle, and new agriculture, the villages were near the river and had many reservoirs around transmission to pose high risk factors. In addition, the most important individual data from previous studies about high-risk groups, those having been infected with OV or eating raw fish were not used to monitor surveillance of re-infected OV cases in the community. Further, the opisthorchiasis control operations lacked the knowledge to integrate at the policy level and participate in control in the community, encountered discontinuity in government support and the activities to reduce OV were slightly concentrated [4].

The primary objective of the present study was to conduct a follow-up on the subjects who were initially enrolled in a prospective study conducted in Southeastern Thailand. The focus was to assess the current reinfection rate of Opisthorchis viverrine (OV). The findings indicate that among the subdistricts, Hua Mueang, a subdistrict of Maha Chana Chai, had the highest prevalence of OV reinfection (14.87%), surpassing the overall reinfection rate observed in the study. The age groups of 25 to 40 years and 41 to 60 years had higher re-infection rates than the age groups of 61 to 75 years. The OV reinfection rate among the elderly was decreasing relevant a population-based cross-sectional study in northeast Thailand reported the infection rates increase across age groups but then reduce after the age of 50 years [16]. The reported in a review article [1] that the prevalence of infection occurs early in life, plateaus after the teen years, and declines in old age. These findings contrast those of related studies whereas, a 1-year study [2] reported a high prevalence of re-infection in the age group of >50 years. The second survey incidence and re-infection rates of OV in upper northeastern communities of Thailand results found the majority of re-infection rates were in the 40 to 60 and over 60 years old male groups [14].

Factors associated with OV reinfection in the present study found income and previous stool examination was significant. In the previous 1-year study, only the previous use of praziquantel was associated with reinfection. However, a cross-sectional study conducted following the implementation of the national strategy [17] identified that five factors were associated with OV reinfection including consumption habits, eating similar to those of family members, history of OV infection, previous use of praziquantel and unsafe disposal of waste food. It is apparent that food behavior is relayed from old to new generations, particularly within families, and some villagers believe that praziquantel can prevent OV infection. A previous study [14] found that the rate of raw fish consumption was 53.2% and that of medicine consumption was10.5%; in a subsequent study [2], the rate of raw fish consumption was 98.5% and the proportion of individuals who believed that praziquantel prevents OV infection was 32.2%. In the present study, the rate of raw fish consumption was found to be the highest (71.5%). The changing economic factors have influenced the current lifestyle of farmers, leading to a transition from the traditional to modern agriculture practices. As they strive to increase their income, farmers often spend less time working on the farm and may take up multiple jobs, which exposes them to new dietary habits and traditional foods. While some farmers are conscious of the risk of OV infection and belong to high- risk groups, they tend to seek health check-ups and use preventive drugs to protect against OV infection by themself, stool examination is not available by health requirement from the public health service, the fecal exam on a routine day by the technician in a health promotion hospital will operate when they having a special campaign or establishing a project to solve the OV problem. However, the study revealed that the proportion of villagers with high and middle-low knowledge regarding OV infection was almost similar at 51.2% and 48.8%, respectively. These findings confirm that villagers may not have sufficient resources to change their high-risk behavior regarding raw fish consumption and may not be concerned that the disease may reoccur after a long time. The limitations of this study were the conducting process in the field took place during a government campaign to reduce OV as part of the implemented national program intervention to villagers, which may have reduced re-infection rates with OV beyond what they really should be.

Despite the best efforts and policies formulated by researchers and experts to eradicate OV infection, the challenge persists due to the deeply ingrained eating behaviors in high-risk communities, where individuals continue to consume raw fish and exhibit high-risk behaviors. As a result, reducing the prevalence of OV infection and re- infection may require a considerable amount of time and sustained efforts. Subjects often re-infected with OV should be screened for CCA and the behavior of consuming raw fish. Further efforts should include programs to enhance improved habits and provide preventive knowledge focusing on high-risk groups and epidemic areas to discourage raw fish consumption.

## Author Contribution Statement

Phubet Saengsawang: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft, Visualization. Phuwasin Buakate: Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Research Group, Thailand.

*Data Availability*

Data is available upon reasonable request.

*Ethical Declaration*

This research approved by ethical review committee for human research faculty of public health, Mahidol university (COA. No. MUPH 2017-161).

*Conflict of Interest*

The authors declare no competing interests.

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