

Prevalence and Associated Factors of *Opisthorchis viverrini* Infection in Nakhon Phanom, Thailand

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Abstract

Background: *Opisthorchis viverrini* infection remains a major public health problem in northeastern Thailand, particularly in communities near freshwater ecosystems with persistent raw fish consumption. Conventional stool-based diagnostics have limited sensitivity, whereas the *O. viverrini*-rapid diagnostic test (OV-RDT) improves infection detection.

Objectives: To determine the prevalence of *O. viverrini* infection and identify factors influencing infection among residents of Nakhon Phanom, Thailand.

Methods: This retrospective cross-sectional analytical study analyzed routine screening data obtained from the Nakhon Phanom Provincial Public Health Office, including 5093 individuals aged ≥ 15 years who were screened for *O. viverrini* infection using OV-RDT during 2023-2024. Prevalence, and associated factors were assessed using multivariable logistic regression.

Results: The overall prevalence of *O. viverrini* infection was 52.5%. The prevalence decreased from 63.0% in 2023 to 35.5% in 2024. Significant factors associated with infection included age 35-59 years (adjusted odds ratios [aOR], 1.15; 95% CI, 1.00-1.33), employment in the agricultural sector (aOR, 1.23; 95% CI, 1.01-1.50), consumption of raw or undercooked freshwater fish (aOR, 2.00; 95% CI, 1.64-2.43), and spatial factors, with residents of Wang Yang district exhibiting the highest risk of infection (aOR, 61.05; 95% CI, 37.38-99.73). The final model explained 23.5% of the variance (pseudo $R^2 = 0.235$).

Conclusions: *O. viverrini* infection remains highly prevalent in Nakhon Phanom, Thailand and is driven by individual, behavioral, and spatial factors, supporting the need for targeted, area-based control and prevention strategies.

Keywords: Prevalence, *Opisthorchis viverrini*, OV-rapid diagnostic test

Citation: Sirikhaeng P, Ungcharoen R. Prevalence and associated factors of *Opisthorchis viverrini* infection in Nakhon Phanom, Thailand. *Res Med J*. 20XX;XX(X):e280013. doi:10.33165/rmj.2027.e280013

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Received: 21 January 2026

Revised: 19 May 2026

Accepted: 15 June 2026

Published Online:

22 June 2026



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Introduction

Opisthorchis viverrini is a food-borne trematode parasite that uses freshwater cyprinid fish as the second intermediate host. Human infection occurs through the consumption of raw or undercooked freshwater fish containing infective metacercariae. After ingestion, the parasites mature in the biliary tract, causing chronic inflammation of the bile ducts and increasing the risk of cholangiocarcinoma.¹⁻³ As a result, *O. viverrini* infection remains an important public health problem in the Greater Mekong Subregion, particularly in Thailand, the Lao People's Democratic Republic, and Cambodia.⁴⁻⁶

Although the national prevalence of *O. viverrini* infection in Thailand has declined over the past decade from 16.1% in 2016 to approximately 3.2% in 2023 high infection rates persist in northeastern Thailand.⁷ Populations living near natural freshwater bodies remain particularly vulnerable due to environmental exposure and traditional dietary practices.^{8,9} Nakhon Phanom province, located in the Songkhram River Basin and the lower Mekong River Basin, is characterized by extensive floodplains and abundant freshwater resources. The continued consumption of raw or undercooked freshwater fish, including traditional dishes such as Koi Pla, Pla Som, and Jaew Bong Pla Ra, contributes substantially to ongoing transmission in this area.¹⁰ Recent surveillance data in Nakhon Phanom province reported a prevalence of *O. viverrini* infection of approximately 22.8% in 2019,¹¹ indicating that infection remains an important public health problem in this area.

The Ministry of Public Health, Thailand has set a target to reduce the prevalence of *O. viverrini* infection to below 1.0% by 2025 and reduce cholangiocarcinoma mortality by two-thirds by 2035 through active surveillance and community-based screening programs.⁷ Conventional stool examination using the modified Kato-Katz technique (MKKT) has been widely used because of its low cost and feasibility. However, its sensitivity is limited, particularly in individuals with low-intensity or chronic infections, and it requires skilled personnel.¹² Recently, a urine-based rapid diagnostic test for *O. viverrini* (OV-rapid diagnostic test [OV-RDT]) has been developed. This test detects parasite antigens within 15-20 minutes, does not require microscopy, and has demonstrated high diagnostic accuracy, with reported sensitivity and specificity of 94.2% and 93.2%, respectively.¹³ These characteristics make OV-RDT suitable for large-scale screening and epidemiological studies.

Most previous studies in Thailand have relied on stool-based diagnostic methods, which may underestimate the true burden of *O. viverrini* infection, especially in individuals with low parasite intensity or asymptomatic chronic infection.^{14,15} Moreover, evidence linking OV-RDT-based diagnosis with demographic characteristics, behavioral risk factors, and spatial context remains limited, and such data are lacking for Nakhon Phanom province.¹³

Therefore, this study aimed to determine the prevalence of *O. viverrini* infection and examine spatially associated factors influencing infection among individuals aged ≥ 15 years in Nakhon Phanom, Thailand, using the OV-RDT. The findings are expected to provide area-specific and clinically relevant evidence to support targeted surveillance, prevention, and control strategies for *O. viverrini* infection in endemic settings.

Methods

A retrospective cross-sectional analytical study was conducted using routine screening data from the Nakhon Phanom Provincial Public Health Office collected between 2023 and 2024. The study aimed to determine the prevalence of *O. viverrini* infection and identify its associated factors among residents of Nakhon Phanom, Thailand. The study population include residents aged ≥ 15 years across all 12 districts who participated in community-based screening programs. *O. viverrini* infections were diagnosed using the OV-RDT.

The sample size adequacy for the multivariable analysis was validated using the events per variable (EPV) criteria. Given 21 parameters and a prevalence of approximately 0.178⁸ included in the final multivariable logistic regression model and 2673 positive cases (events) identified from the total of 5093 participants, the study achieved an EPV of approximately 127. This significantly exceeds the widely recommended minimum threshold

of 10 EPV, thereby ensuring excellent model stability, reducing the standard errors of the estimates, and eliminating the potential risk of model overfitting.

Strict data cleaning protocols were applied; duplicate records were identified via unique national identification numbers and excluded prior to analysis to maintain the independence of observations. Each participant was represented only once in the final dataset. After excluding incomplete records, a total of 5093 residents were included in the analysis, consisting of 2673 positive cases (including all intensity levels from 1+ to 4+) and 2420 negative cases.

Data were extracted using a standardized electronic form and checked for completeness and consistency. The dependent variable was defined by the *O. viverrini* infection status. Independent variables included demographic characteristics (sex, age, education, and occupation), behavioral factors (consumption of raw or undercooked freshwater fish), and geographical/environmental factors (district of residence and household proximity to natural water sources).

Prevalence was reported as percentages, while demographic and behavioral characteristics were summarized using frequencies and percentages. Factors associated with *O. viverrini* infection were initially screened using univariable logistic regression. Variables with a *P* value < .25 and those considered epidemiologically relevant were entered into the multivariable logistic regression model. Multicollinearity was assessed using the variance inflation factor (VIF), and variables with a VIF ≥ 5 were excluded. Multivariable analysis was performed using a backward stepwise selection approach while retaining key epidemiological variables, including sex, age, education, and occupation, to control for potential confounding effects. The environmental variable, household proximity to natural water sources, was examined in univariable analyses but was excluded from the final multivariable model due to model estimation and convergence problems resulting from its high correlation with certain geographic localities. The results were presented as adjusted odds ratio (aOR) with 95% CI, and statistical significance was set at *P* value < .05.

To visualize geographic variation, the district-level prevalence of *O. viverrini* infection was mapped. Prevalence data were aggregated by district and displayed through choropleth mapping techniques. Provincial administrative boundary shapefiles were generated using Quantum Geographic Information System (QGIS) version 3.40.1¹⁶ (Figure 1). Prevalence values were categorized into 5 classes using the Jenks natural breaks classification method for visualization purposes.

Results

A total of 5093 individuals aged ≥ 15 years in Nakhon Phanom province were screened for *O. viverrini* infection using OV-RDT during 2023-2024. Overall, 2673 individuals tested positive, yielding a prevalence of 52.5%. Surveillance data showed a declining trend, with prevalence decreasing from 63.0% in 2023 to 35.5% in 2024.

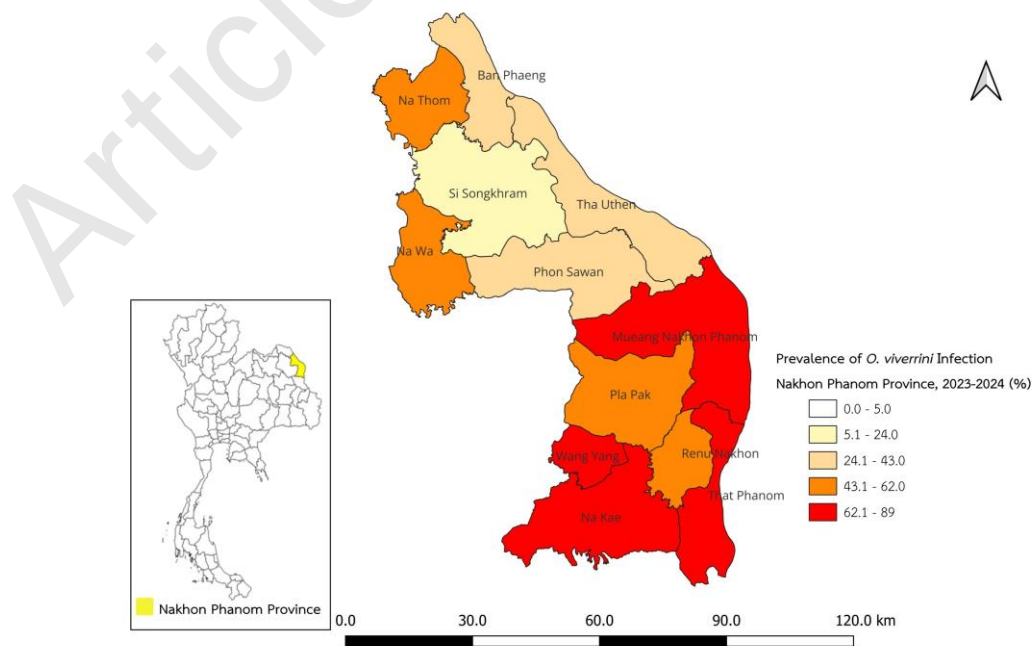
The prevalence of *O. viverrini* infection varied across districts (Table 1 and Figure 1). The highest prevalence was observed in Wang Yang district (88.9%), followed by the districts of That Phanom (71.8%), Mueang Nakhon Phanom (70.8%), and Na Kae (70.3%), whereas Si Songkhram district had the lowest prevalence (12.5%). Prevalence was similar between males (52.1%) and females (52.7%). Most infected individuals were aged ≥ 35 years, accounting for 92.7% of cases, with the highest proportion in the 35-59 years

age group. Infection was observed across all educational levels, with the highest prevalence among individuals with tertiary education (57.5%), followed by those with primary education (52.9%) and secondary education (50.7%). Prevalence was comparable between the nonagricultural and agricultural sectors (54.1% vs 52.3%) (Table 2).

Table 1. Distribution of *Opisthorchis viverrini* Infection by District in Nakhon Phanom, Thailand, Using OV-RDT, 2023-2024

District	No. (%)		95% CI
	Not Infected	Infected	
Tha Uthen	229 (71.6)	91 (28.4)	0.23-0.33
That Phanom	196 (28.2)	498 (71.8)	0.68-0.75
Na Thom	159 (45.4)	191 (54.6)	0.49-0.60
Na Wa	183 (54.6)	152 (45.4)	0.40-0.51
Na Kae	224 (29.7)	531 (70.3)	0.67-0.74
Ban Phaeng	239 (68.1)	112 (31.9)	0.27-0.37
Pla Pak	161 (50.0)	161 (50.0)	0.45-0.56
Wang Yang	34 (11.1)	271 (88.9)	0.85-0.92
Si Songkhram	280 (87.5)	40 (12.5)	0.09-0.16
Mueang Nakhon Phanom	93 (29.2)	225 (70.8)	0.66-0.76
Renu Nakhon	147 (46.2)	171 (53.8)	0.48-0.59
Phon Sawan	475 (67.4)	230 (32.6)	0.29-0.36
Total	2420 (47.5)	2673 (52.5)	0.51-0.54

Figure 1. Spatial Distribution of *Opisthorchis viverrini* Infection by District in Nakhon Phanom, Thailand, Using OV-RDT, 2023-2024



Abbreviation: OV-RDT, *O. viverrini*-rapid diagnostic test.

Individuals who reported consumption of raw or undercooked freshwater fish had a higher prevalence of *O. viverrini* infection than those who did not report such consumption (55.9% vs 31.4%, respectively; Table 2). Regarding environmental exposure, individuals residing near the Kam River exhibited a high prevalence of infection (71.9%), whereas those living near the Songkhram River had the lowest prevalence (12.5%) (Table 2). These environmental variables were examined in univariable analyses. However, they were excluded from the final multivariable model because of model estimation and convergence problems.

The results of the multivariable logistic regression analysis are presented (Table 2). The adjusted logistic regression model included sex, age group, educational level, occupation, consumption of raw or undercooked freshwater fish, and district of residence. Age, occupation, consumption of raw or undercooked freshwater cyprinid fish, and district of residence were independently associated with *O. viverrini* infection. Individuals aged 35-59 years had higher odds of infection than those aged ≥ 60 years (aOR, 1.15; 95% CI, 1.00-1.33; $P = .045$). Agricultural workers were more likely to be infected than nonagricultural workers (aOR, 1.23; 95% CI, 1.01-1.50; $P = .037$). Consumption of raw or undercooked freshwater cyprinid fish was strongly associated with infection, with individuals reporting this behavior having approximately twice the odds of infection compared with nonconsumers (aOR, 2.00; 95% CI, 1.64-2.43; $P < .001$).

With respect to spatial factors, the district of residence was the strongest predictor of infection. Compared with residents of Si Songkhram district (reference group), individuals living in Wang Yang district had markedly higher odds of infection (aOR, 61.05; 95% CI, 37.38-99.73; $P < .001$). Elevated odds were also observed among residents of That Phanom (aOR, 17.65; 95% CI, 12.17-25.60), Na Kae (aOR, 16.47; 95% CI, 11.38-23.83), and Mueang Nakhon Phanom districts (aOR, 16.05; 95% CI, 10.63-24.24), all of which were statistically significant ($P < .001$). Overall, the final multivariable model explained 23.5% of the variance in *O. viverrini* infection (pseudo $R^2 = 0.235$).

Table 2. Prevalence and Factors Associated With *Opisthorchis viverrini* Infection Among Individuals Aged ≥ 15 Years in Nakhon Phanom, Thailand, 2023-2024

in Nakorn Phanom, Thailand, 2023-2024						
Variable	No. (%)		cOR (95% CI)	P Value	aOR (95% CI)	P Value
	Not Infected	Infected				
Sex						
Male	963 (47.9)	1047 (52.1)	0.97 (0.87-1.09)	.649	0.96 (0.85-1.09)	.515
Female	1457 (47.3)	1626 (52.7)	1 [Reference]	NA	1 [Reference]	NA
Age, y						
< 35	223 (53.3)	195 (46.7)	0.83 (0.67-1.03)	.096	1.16 (0.89-1.50)	.272
35-59	1450 (46.1)	1693 (53.9)	1.11 (0.98-1.26)	.091	1.15 (1.00-1.33)	.045
≥ 60	747 (48.8)	785 (51.2)	1 [Reference]	NA	1 [Reference]	NA
Educational level						
Primary	1709 (47.1)	1918 (52.9)	0.83 (0.61-1.13)	.237	0.87 (0.62-1.28)	.527
Secondary	637 (49.3)	655 (50.7)	0.76 (0.55-1.05)	.094	0.78 (0.54-1.13)	.188
Tertiary	74 (42.5)	100 (57.5)	1 [Reference]	NA	1 [Reference]	NA
Occupation						
Agricultural sectors	2131 (47.7)	2333 (52.3)	0.93 (0.79-1.10)	.403	1.23 (1.01-1.50)	.037
Nonagricultural sectors	289 (45.9)	340 (54.1)	1 [Reference]	NA	1 [Reference]	NA

Table 2. Prevalence and Factors Associated With *Opisthorchis viverrini* Infection Among Individuals Aged ≥ 15 Years in Nakhon Phanom, Thailand, 2023-2024 (Continued)

Variable	No. (%)		cOR (95% CI)	P Value	aOR (95% CI)	P Value
	Not Infected	Infected				
Consumption of raw or undercooked freshwater fish						
Yes	1938 (44.1)	2452 (55.9)	2.76 (2.33-3.27)	< .001	2.00 (1.64-2.43)	< .001
No	482 (68.6)	221 (31.4)	1 [Reference]	NA	1 [Reference]	NA
District of residence						
Si Songkhram	280 (87.5)	40 (12.5)	1 [Reference]	NA	1 [Reference]	NA
Tha Uthen	229 (71.6)	91 (28.4)	2.78 (1.85-4.20)	< .001	3.35 (2.21-5.08)	< .001
That Phanom	196 (28.2)	498 (71.8)	17.79 (12.28-25.76)	< .001	17.65 (12.17-25.60)	< .001
Na Kae	224 (29.7)	531 (70.3)	16.60 (11.51-23.93)	< .001	16.47 (11.38-23.83)	< .001
Na Thom	159 (45.4)	191 (54.6)	8.41 (5.68-12.45)	< .001	8.88 (5.98-13.20)	< .001
Na Wa	183 (54.6)	152 (45.4)	5.81 (3.92-8.63)	< .001	5.60 (3.76-8.32)	< .001
Ban Phaeng	239 (68.1)	112 (31.9)	3.28 (2.20-4.89)	< .001	3.67 (2.44-5.50)	< .001
Pla Pak	161 (50.0)	161 (50.0)	7.00 (4.71-10.41)	< .001	8.50 (5.67-12.73)	< .001
Phon Sawan	475 (67.4)	230 (32.6)	3.39 (2.35-4.89)	< .001	3.70 (2.56-5.35)	< .001
Mueang Nakhon Phanom	93 (29.2)	225 (70.8)	16.94 (11.24-25.52)	< .001	16.05 (10.63-24.24)	< .001
Renu Nakhon	147 (46.2)	171 (53.8)	8.14 (5.47-12.12)	< .001	8.32 (5.58-12.42)	< .001
Wang Yang	34 (11.1)	271 (88.9)	55.79 (34.29-90.78)	< .001	61.05 (37.38-99.73)	< .001
Household proximity to natural water source						
Kam River	574 (28.1)	1,470 (71.9)	4.18 (3.62-4.83)	< .001	NA	NA
Nam Un River	183 (54.6)	152 (45.4)	1.36 (1.07-1.73)	.013	NA	NA
Songkhram River	280 (87.5)	40 (12.5)	0.23 (0.17-0.33)	< .001	NA	NA
Mekong River	509 (51.7)	476 (48.3)	1.53 (1.30-1.80)	< .001	NA	NA
No proximity to natural water bodies	874 (62.0)	535 (38.0)	1 [Reference]	NA	NA	NA

Abbreviations: aOR, adjusted odds ratio; cOR, crude odds ratio; NA, not applicable.

Discussion

The findings of this study indicate that *O. viverrini* infection among individuals aged ≥ 15 years in Nakhon Phanom province remains alarmingly high, with an overall prevalence of 52.5%. This prevalence greatly exceeds the Ministry of Public Health target of reducing opisthorchiasis prevalence to $\leq 1.0\%$ by 2025 and is substantially higher than the national prevalence reported in 2023 (3.2%).⁷ The prevalence observed was also higher than reports from northeastern Thailand and the Greater Mekong Subregion, where a 2024 review reported an overall prevalence of 21.11%,⁸ while prevalence rates of 12.4% and 7.01% were reported in Khon Kaen province in 2024,¹⁷ and Sakon Nakhon province in 2020,¹⁰ respectively. However, these comparisons should be interpreted cautiously because most national and regional surveys used stool examination methods, whereas this study employed OV-RDT, which has higher diagnostic sensitivity and may detect active or recent infections in individuals with low parasite burdens.¹³ This methodological difference may partly explain the higher prevalence observed. Nevertheless, the findings suggest that current control and prevention measures may remain insufficient to interrupt transmission, despite the declining trend observed during 2023-2024. The persistently

high prevalence reflects continued exposure to environmental and behavioral risk factors within endemic communities.

The detection of infection in both sexes may reflect the cultural context of shared household dining practices in Nakhon Phanom province. After controlling for potential confounders, sex was not significantly associated with *O. viverrini* infection. This finding contrasts with previous localized or district-level studies, such as those by Chuangchaiya et al¹⁸ and Srithai et al¹¹ which reported a higher risk among males. One possible explanation may relate to differences in spatial scale. In microlevel or district-level studies, sex-specific differences in risk behaviors may be more apparent, whereas province-wide analyses may reduce the influence of localized individual behaviors. In the present study, shared intrahousehold food consumption practices may have contributed to similar exposure levels between males and females, thereby reducing observable differences in infection prevalence at the provincial level. However, household-level clustering data and shared dietary behavior information were not available to directly evaluate this hypothesis.

Regarding age, *O. viverrini* infection was observed across all age groups, with individuals aged 35-59 years demonstrating significantly higher odds of infection than those aged ≥ 60 years. This finding differs from some previous studies in Thailand that identified older age groups as having the highest risk of infection.¹¹ One possible explanation is that individuals in the working-age group may experience more frequent exposure to risk behaviors, particularly participation in social gatherings and regular consumption of traditional dishes made with raw or undercooked freshwater fish. In addition, working-age adults in Nakhon Phanom province may remain actively involved in agricultural and fishing-related activities associated with freshwater environments, thereby increasing opportunities for repeated exposure. The findings also suggest that infection risk may reflect cumulative behavioral and environmental exposure sustained throughout adulthood rather than age alone.

Although educational level was not significantly associated with *O. viverrini* infection, consistent with previous studies in Thailand,^{11, 18} individuals with tertiary education demonstrated the highest prevalence of infection (57.5%). This finding suggests that higher educational attainment alone may be insufficient to modify deeply rooted sociocultural dietary behaviors in Nakhon Phanom province, particularly the consumption of raw or undercooked freshwater fish. Many highly educated individuals may remain lifelong residents of endemic communities and continue participating in traditional household food-sharing practices. The persistence of infection among highly educated individuals thus highlights a gap between health knowledge and preventive practices, reflecting the strong influence of local food culture and long-standing social norms.¹⁹

Regarding occupation, individuals in agricultural sectors had significantly higher odds of *O. viverrini* infection than those in nonagricultural sectors after adjustment for covariates, consistent with the systematic review by Pengput et al,²⁰ Although the crude prevalence was slightly higher among nonagricultural sectors (54.1%), this pattern likely reflects confounding from district-level endemicity and shared raw fish consumption behaviors. After adjustment, the increased risk among agricultural sectors may relate to greater exposure to freshwater environments and traditional fishing-related activities. These findings suggest that infection risk is influenced by the combined effects of geographic context, dietary behaviors, and shared sociocultural lifestyles within endemic communities.

Consumption of raw or undercooked freshwater fish emerged as the strongest risk factor for *O. viverrini* infection. Individuals reporting this behavior had approximately

twice the odds of infection compared with those who did not. This finding is strongly supported by epidemiological evidence identifying traditional raw freshwater fish dishes as the principal route of transmission in northeastern Thailand.¹⁰ Despite long-standing public health campaigns, these dietary practices persist, underscoring the need for culturally sensitive and context-specific behavioral interventions.

The findings demonstrated marked district-level geographic variation in *O. viverrini* infection across Nakhon Phanom province. Wang Yang district exhibited the highest risk of infection, followed by That Phanom, Na Kae, and Mueang Nakhon Phanom districts, whereas Si Songkhram district had the lowest prevalence. Most high-risk districts are connected to the Kam River and Mekong River ecosystems, which may support the persistence of the parasite life cycle through intermediate hosts. These areas are also associated with traditional fishing lifestyles, reliance on freshwater resources, and consumption of raw or undercooked freshwater fish. In contrast, the lower prevalence in Si Songkhram district may reflect differences in environmental conditions and sociocultural contexts. Although individual-level behavioral data were available, detailed environmental and community-level information was not collected. These findings are consistent with previous studies showing that freshwater ecosystems, proximity to river basins, and traditional raw fish consumption contribute to the geographic distribution of *O. viverrini* infection in northeastern Thailand and the Greater Mekong region.^{9, 18}

This study has several limitations. The cross-sectional design limits causal and temporal interpretation of the observed associations. In addition, the study population was derived from participants in a provincial screening program and may not fully represent the general population. Although several significant associated factors were identified, the moderate model fit suggests that additional environmental and community-level determinants may also contribute to infection risk. Furthermore, caution is required when generalizing the findings to other geographic or sociocultural settings because the study was conducted in a single endemic province in northeastern Thailand. Future longitudinal and multicenter studies are needed to better understand the transmission dynamics and geographic variation of *O. viverrini* infection.

Conclusions

The findings of this study indicate that *O. viverrini* infection remains a major public health concern in Nakhon Phanom province, particularly in districts connected to major freshwater ecosystems and among communities with persistent raw fish consumption behaviors. Prevention and control measures should prioritize high-risk districts and emphasize culturally tailored behavioral interventions targeting traditional raw fish consumption. Strengthening community-based screening, surveillance, and health education in endemic areas may help reduce ongoing transmission. In addition, control strategies should integrate geographic context, environmental exposure, and local sociocultural practices to improve long-term *O. viverrini* prevention and control.

Additional Information

Acknowledgments: The author would like to express sincere gratitude to the Nakhon Phanom Provincial Public Health Office for providing the data for this study. Appreciation is also extended to the Faculty of Public Health, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, as well as to the experts and all individuals involved for their valuable support, guidance, and contributions that made this study possible.

Ethics Approval: This study was approved by the Human Research Ethics Committee of the Nakhon Phanom Provincial Public Health Office, Thailand (RCE 100/68 on 15 August 2025).

Clinical Trial Consideration: This study does not report on a clinical trial.

Financial Support: No financial support was provided for this study.

Conflict of Interest: The authors declare no conflict of interest.

Declaration of AI Assistance: The authors used generative AI tools, ChatGPT and Gemini, in the preparation of this manuscript. The AI tools were utilized for language refinement, including grammar and clarity. All AI-assisted content was thoroughly reviewed and verified by the authors to ensure accuracy, validity, and integrity. The authors take full responsibility for the content presented.

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