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Factors associated with opisthorchiasis preventive behavior in a rural endemic community of northeastern Thailand: A cross-sectional study

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ABSTRACT

Objective: To identify factors associated with preventive behaviour against opisthorchiasis among adults in a rural endemic community in northeastern Thailand.

Methods: A community-based cross-sectional study was conducted in Muang Sri Kai Subdistrict, Ubon Ratchathani Province, Thailand, during September to October 2023 and selected adults using simple random sampling. We collected data on sociodemographic characteristics, knowledge, attitudes, previous stool examination, and preventive behaviour using a structured, self-administered questionnaire. Associations were analyzed using Pearson's *Chi-square* or exact tests, as appropriate, and binary logistic regression, with adjusted odds ratios (aORs) and 95% confidence intervals.

Results: The analysis included 339 adults. The mean age of participants was (45.4±8.8) years, and 64.0% were female. A previous stool examination was reported by 81.4% of participants, and 81.1% reported consuming raw food. Overall, 296 participants (87.3%) demonstrated adequate preventive behavior. Previous stool examination (aOR 2.82, 95% CI 1.35-5.89), higher knowledge score (aOR 1.27 per point, 95% CI 1.13-1.43), and more favorable attitude score (aOR 2.76 per point, 95% CI 1.51-5.02) were associated with increased odds of adequate preventive behavior.

Conclusions: Knowledge, attitudes, and previous stool examination were associated with adequate preventive behavior. The cross-sectional design limits causal inference, and previous screening may indicate broader engagement with health services. Future prospective studies should assess integrated screening and culturally appropriate preventive education.

KEYWORDS: *Opisthorchis viverrini*; Opisthorchiasis; Preventive behavior; Knowledge; Attitude; Endemic community; Thailand

1. Introduction

Opisthorchiasis, caused by *Opisthorchis (O.) viverrini*, remains an important food-borne parasitic disease in Southeast Asia, particularly in Thailand, Cambodia, Lao PDR, Myanmar, and southern Vietnam, where it continues to be a major public health concern. Transmission

Summary

Question: What factors are associated with adequate opisthorchiasis preventive behavior among adults in a rural endemic community in northeastern Thailand?

Findings: In this community-based cross-sectional study of 339 adults, 296 (87.3%) had adequate preventive behavior. Higher knowledge scores, more favorable attitudes, and prior stool examination were significantly associated with adequate preventive behavior.

Meaning: Knowledge, attitudes, and previous stool examination were associated with preventive behavior, while prospective studies are needed to clarify temporal relationships and evaluate preventive interventions.

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is strongly associated with the traditional consumption of raw, undercooked, or fermented freshwater cyprinid fish, especially among communities living along the Mekong River. These fish serve as intermediate hosts for *O. viverrini* when improperly prepared, thereby sustaining the parasite life cycle and ongoing human infection[1-4]. Although the biological pathway of transmission is well established, continued endemicity is also influenced by human behavior, dietary customs, and socio-cultural practices. Despite decades of public health campaigns, culturally embedded food practices remain common in many endemic communities, highlighting the difficulty of achieving sustained behavioral change.

Consumption of contaminated fish is common in endemic settings and contributes to the high burden of opisthorchiasis and related hepatobiliary disease in the region. Chronic *O. viverrini* infection is associated with biliary inflammation, periductal fibrosis, cholangitis, and an increased risk of cholangiocarcinoma[5-7,12]. This continuing disease burden reflects not only environmental and biological exposure, but also persistent behavioral risk factors that maintain transmission. Importantly, awareness of infection risk does not always translate into protective behavior, particularly when traditional dietary practices are deeply rooted in daily life.

Northeastern Thailand is one of the most affected endemic areas, with a high prevalence of *O. viverrini* infection and one of the highest incidences of cholangiocarcinoma worldwide[6,10,11]. Opisthorchiasis is often asymptomatic in its early stages and may remain undetected for years, allowing chronic infection and progressive hepatobiliary complications to develop[12,13]. In Thailand, consumption of raw fish dishes and pla som (traditional fermented fish) remains common and continues to be a possible source of infection[14]. Previous studies have reported persistent infection in several provinces of northeastern Thailand, including Chaiyaphum and Khon Kaen, where raw fish consumption remains frequent[15,16]. These findings indicate that biomedical knowledge and access to treatment alone may be insufficient to interrupt transmission without corresponding preventive behavior.

In rural endemic regions of northeastern Thailand, adults are an important population because they frequently participate in social and household food practices that may increase exposure through routine dietary behaviors. Addressing prevention gaps requires not only documenting exposure patterns but also examining how knowledge, attitudes, and prior health-service experiences influence preventive practices. Evidence on factors associated with opisthorchiasis preventive behavior among adults in rural endemic communities remains limited. The Knowledge-Attitude-Practice framework provides a useful basis for examining relationships among knowledge, attitudes, and preventive practices.

Evidence regarding the independent associations of cognitive and health-service-related factors with opisthorchiasis preventive

behavior in rural endemic communities remains limited, particularly regarding whether previous stool examination is associated with preventive behavior after accounting for knowledge and attitudes. Understanding these associations may help identify research priorities and inform the development of community-based prevention programs. Accordingly, this study examined associations between knowledge, attitudes, previous stool examination, selected sociodemographic characteristics, and opisthorchiasis preventive behavior among adults in a rural endemic community in northeastern Thailand.

2. Methods

2.1. Study design and setting

This community-based cross-sectional analytical study examined factors associated with opisthorchiasis preventive behavior among adults in a rural endemic community in northeastern Thailand. The cross-sectional design enabled the assessment of associations between sociodemographic characteristics, knowledge, attitudes, previous health-service experiences, and preventive behavior at a single point in time.

Data were collected between September and October 2023 in Muang Sri Kai Subdistrict, Warin Chamrap District, Ubon Ratchathani Province, Thailand, an area where opisthorchiasis remains a public health concern. The study population comprised adults residing in the community during the study period.

Data were collected using a structured self-administered questionnaire covering sociodemographic characteristics, knowledge regarding opisthorchiasis, attitudes toward disease prevention, and self-reported preventive behavior. Participants completed the questionnaire independently under standardized conditions. The average completion time was approximately 10-15 min.

2.2. Sample size and participant recruitment

The source population comprised approximately 2836 adults residing in Muang Sri Kai Subdistrict. We calculated the required sample size as 339 participants[19]. The final analytical sample comprised 339 participants selected from official community population lists using simple random sampling. All 339 participants provided written informed consent, completed the questionnaire, and had complete data for the variables included in the analysis. Detailed counts for intermediate recruitment stages, such as individuals contacted, unavailable, ineligible, or declining participation, were not available in the recruitment records used for this analysis; therefore, we could not reliably calculate a stage-specific response rate.

2.3. Participants

The source population comprised approximately 2836 adults residing in Muang Sri Kai Subdistrict. Researchers selected participants from official community population lists using simple random sampling. Simple random sampling reduced selection bias within the eligible sampling frame.

Eligibility criteria included adults aged 30 and over of either sex who had resided in the study area for at least six months and could read and write Thai. We excluded individuals who were unavailable after two follow-up visits or unwilling to participate. We invited eligible individuals to participate and obtained written informed consent before data collection. We included 339 participants in the final analysis.

Because data collection relied on a self-administered questionnaire, eligibility was limited to individuals who could read and write Thai. This criterion may have excluded community members with limited literacy and should be considered when interpreting the study's representativeness and generalizability.

2.4. Data collection instruments

Data were collected using a structured, self-administered questionnaire adapted from previously published studies on opisthorchiasis and knowledge-attitude-practice constructs[14,16,21,22]. The questionnaire was developed in Thai for administration to Thai-speaking adults in the study area; therefore, cross-language translation and back-translation were not required. Items were further adapted to reflect the local epidemiological and sociocultural context of opisthorchiasis in rural northeastern Thailand.

The questionnaire comprised four sections: (1) sociodemographic and health-related characteristics; (2) 21 items assessing knowledge of opisthorchiasis; (3) 13 items assessing attitudes toward opisthorchiasis prevention; and (4) 15 items assessing preventive behavior. The complete questionnaire used for data collection is provided in the Supplementary Material.

2.5. Measurement and scoring of study constructs

We assessed knowledge of opisthorchiasis using 21 dichotomous items. Correct responses were scored as 1 and incorrect responses as 0, yielding a total score ranging from 0 to 21. Total scores were classified as low (0-12), moderate (13-16), or high (17-21) according to the predefined criteria[17].

We assessed attitudes toward opisthorchiasis prevention using 13 items rated on a five-point Likert scale, from 1 (strongly disagree) to 5 (strongly agree). To ensure that higher scores consistently represented more favorable preventive attitudes, negatively worded

or risk-supportive items (Items 1, 4, 6, 7, 8, 9, 10, 11, and 12) were reverse-scored (1=5, 2=4, 3=3, 4=2, and 5=1). Mean scores were calculated across the 13 items and classified as low (1.00-2.33), moderate (2.34-3.66), or high (3.67-5.00)[18].

We assessed preventive behavior regarding opisthorchiasis using 15 items covering food-related practices, hygiene, food handling, stool examination, and anthelmintic treatment. Responses were rated on a three-point frequency scale: regularly (6-7 days per week), occasionally (3-4 days per week), and never. For positively worded preventive items, responses were scored as 3, 2, and 1, respectively. Item 4, which assessed consumption of raw fermented fish (Pla-ra) or raw jaew bong containing fermented fish, was a risk behavior and was reverse-scored (regularly = 1, occasionally = 2, and never = 3), so higher scores consistently represented more favorable preventive behavior. We calculated the mean score across the 15 items and rounded it to two decimal places before applying the predefined categories: low (1.00-1.66), moderate (1.67-2.33), and high (2.34-3.00)[18]. For the primary binary analysis, we combined the low and moderate categories as suboptimal preventive behavior, while classifying the high category as adequate preventive behavior.

2.6. Development, content validation, pilot testing, and reliability assessment of the questionnaire

Three experts in public health and parasitology assessed content validity using the Index of Item-Objective Congruence (IOC). We revised items identified as needing refinement based on expert feedback before field implementation. The finalized questionnaire for data collection included 21 knowledge items, 13 attitude items, and 15 preventive behavior items. Pilot testing was conducted with 30 individuals possessing characteristics similar to the target population. Internal consistency was evaluated using the Kuder-Richardson Formula 20 (KR-20) for the 21 dichotomously scored knowledge items and Cronbach's alpha for the attitude and preventive-behavior scales. The reliability coefficients were 0.813 for knowledge, 0.701 for attitude, and 0.754 for preventive behavior. The complete questionnaire is available in the Supplementary Material.

2.7. Data analysis

We analyzed data using IBM SPSS Statistics. Continuous variables were summarized using means and standard deviations (SDs) together with observed minimum and maximum values. Categorical variables were summarized using frequencies and percentages. Complete data were available for all variables included in the analyses.

We examined the distribution of preventive-behavior scores before inferential analysis. Scores were concentrated toward the upper end

of the scale, indicating limited variability and a ceiling effect. Based on the predefined classification criteria, preventive behavior was categorized as low, moderate, or high. Mean preventive-behavior scores were rounded to two decimal places before applying the predefined category boundaries. For the primary binary analysis, the low and moderate categories were combined as suboptimal preventive behavior, whereas the high category was classified as adequate preventive behavior.

Associations between categorical variables and preventive behavior were examined using Pearson's *Chi*-square test when expected cell-frequency assumptions were satisfied. Expected cell counts were assessed for each contingency table. Fisher's exact test was used for 2×2 tables when expected cell frequencies were insufficient for reliable application of the Pearson *Chi*-square approximation, whereas a Monte Carlo exact procedure was used for larger contingency tables when appropriate. Frequencies and row percentages are reported. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

For the primary multivariable analysis, we used binary logistic regression, with adequate preventive behavior coded as 1 and suboptimal preventive behavior as 0. Because the less frequent outcome category comprised only 43 participants, a parsimonious model was used to reduce the risk of overfitting. Previous stool examination, knowledge score, and corrected attitude score were included based on substantive and epidemiological considerations rather than solely on bivariate P values. We entered previous stool examination as a binary variable, with never having undergone stool examination as the reference category, and entered knowledge and corrected attitude scores as continuous variables.

We assessed multicollinearity using variance inflation factors (VIFs) and evaluated the linearity-in-the-logit assumption for the continuous predictors. Potentially influential observations were examined using standardized residuals, leverage values, and Cook's distance. We assessed overall model significance using the likelihood-ratio test, model calibration using the Hosmer-Lemeshow goodness-of-fit test, and discrimination using the area under the receiver operating characteristic curve (AUC). Adjusted odds ratios (aORs), 95% confidence intervals (CIs), regression coefficients (β), standard errors (SEs), and P values were reported.

Sensitivity analyses were conducted using alternative representations of the preventive-behavior outcome. First, the corrected mean preventive-behavior score was analyzed as a continuous outcome using linear regression with heteroscedasticity-robust standard errors. Second, the original low, moderate, and high preventive-behavior categories were analyzed using ordinal logistic regression. Both sensitivity analyses included the same predictors as the primary model: previous stool examination, knowledge score, and corrected attitude score. Formal assessment of the proportional-odds assumption for the ordinal model was limited by the extreme

sparsity of the low preventive-behavior category, which contained only one participant. Therefore, we treated the ordinal model as a supplementary sensitivity analysis and interpreted it with caution.

2.8. Potential sources of bias and bias reduction

We implemented several measures to reduce potential sources of bias. Participants were selected using simple random sampling from official community population lists to reduce selection bias within the eligible sampling frame. Participants completed the self-administered questionnaires under standardized conditions to minimize interviewer-related variation. We collected responses anonymously to reduce concerns about social desirability bias. In addition, the questionnaire underwent expert content review and pilot testing before field implementation to improve the clarity and appropriateness of the study measures.

Nevertheless, several potential sources of bias remained. Self-reported preventive behaviors and previous exposure histories may have been affected by recall and social desirability bias. The eligibility requirement that participants be able to read and write Thai may have excluded individuals with lower literacy, potentially introducing selection bias and limiting sample representativeness. Previous stool examination and raw-food consumption were measured using broad self-reported categories, which may have resulted in exposure misclassification and residual confounding. Furthermore, because of the cross-sectional design, we could not establish the temporal sequence between previous exposures and preventive behavior.

2.9. Ethical approval

Ethical approval was obtained from the Ethics Review Committee for Human Research, Sirindhorn College of Public Health, Ubon Ratchathani, Thailand (COA No. SCPHUB S021-2022; approved on 21 September 2022). The study followed the ethical principles of the Declaration of Helsinki. We obtained written informed consent from all participants before enrollment. The study ensured confidentiality and voluntary participation throughout.

3. Results

3.1. General characteristics of participants

The analysis included 339 participants with complete data for all variables examined. Most participants were female (64.0%), and the mean age was (45.4±8.8) years. More than half were farmers (53.4%), and 91.4% reported a monthly income of ≤10000 THB. 81.1% of participants reported a history of raw-food consumption.

Most participants had no underlying disease (78.2%). Overall, 81.4% reported having previously undergone stool examination, whereas 18.6% reported never having undergone stool examination (Table 1).

Table 1. Participants characteristics ($n=339$).

Category	n (%)
Sex	
Male	122 (36.0)
Female	217 (64.0)
Age, years, mean $\pm$ SD	45.4 $\pm$ 8.8
Age, years	
≤ 45	179 (52.8)
≥ 46	160 (47.2)
Marital status	
Single	102 (30.1)
Married	237 (69.9)
Occupation	
Farmer	181 (53.4)
Non-farmer	158 (46.6)
Monthly income, THB	
≤ 10000	310 (91.4)
≥ 10001	29 (8.6)
Education level	
Primary school	181 (53.4)
Higher than primary school	158 (46.6)
History of stool examination	
Ever	276 (81.4)
Never	63 (18.6)
Raw fish consumption	
Ever	275 (81.1)
Never	64 (18.9)
Underlying disease	
None	265 (78.2)
Present	74 (21.8)

Data are presented as n (%) unless otherwise indicated.

3.2. Knowledge, attitudes, and preventive behavior

Table 2 summarises the distributions of knowledge, attitudes, and preventive behavior regarding opisthorchiasis. The mean knowledge score was 15.99 ± 2.60 (range: 7.00–21.00); 160 participants (47.2%) had high knowledge, 153 (45.1%) moderate, and 26 (7.7%) low. The mean attitude score was 3.67 ± 0.60 (range: 2.23–5.00); 180 participants (53.1%) had a high attitude level, 155 (45.7%) had a moderate level, and 4 (1.2%) had a low level. The mean preventive-behavior score was 2.56 ± 0.20 (range: 1.27–2.93). One participant (0.3%) had low preventive behavior, 42 (12.4%) had moderate preventive behavior, and 296 (87.3%) had high preventive behavior. For the primary binary analysis, the low and moderate categories were combined, resulting in 43 participants (12.7%) with suboptimal preventive behavior and 296 (87.3%) with adequate preventive behavior (Table 2).

Table 2. Distribution of knowledge, attitudes, and preventive behavior regarding opisthorchiasis ($n=339$).

Variables	Score range	n (%)
Knowledge, mean $\pm$ SD	15.99 $\pm$ 2.60	
Low	0–12	26 (7.7)
Moderate	13–16	153 (45.1)
High	17–21	160 (47.2)
Attitude, mean $\pm$ SD	3.67 $\pm$ 0.60	
Low	1.00–2.33	4 (1.2)
Moderate	2.34–3.66	155 (45.7)
High	3.67–5.00	180 (53.1)
Preventive behavior, mean $\pm$ SD	2.56 $\pm$ 0.20	
Low	1.00–1.66	1 (0.3)
Moderate	1.67–2.33	42 (12.4)
High	2.34–3.00	296 (87.3)
Suboptimal (low + moderate)	1.00–2.33	43 (12.7)
Adequate (high)	2.34–3.00	296 (87.3)

3.3. Association between knowledge, attitudes, and preventive behavior

Knowledge level was significantly associated with preventive behavior (Monte Carlo exact $P < 0.001$). The proportion of participants with adequate preventive behavior increased from 61.5% among those with low knowledge to 86.9% among those with moderate knowledge and 91.9% among those with high knowledge. Attitude level showed a borderline association with preventive behavior (Monte Carlo exact $P = 0.05$). This finding should be interpreted cautiously because the low-attitude category included only four participants, resulting in a small expected cell count (Table 3).

Table 3. Association between knowledge, attitudes, and opisthorchiasis preventive behavior ($n=339$).

Variables	Suboptimal ($n=43$)	Adequate ($n=296$)	P
Knowledge			
Low	10 (38.5)	16 (61.5)	<0.001
Moderate	20 (13.1)	133 (86.9)	
High	13 (8.1)	147 (91.9)	
Attitude			
Low	0 (0.0)	4 (100.0)	0.05
Moderate	27 (17.4)	128 (82.6)	
High	16 (8.9)	164 (91.1)	

Notes: Values are presented as n (row %). We examined expected cell frequencies before selecting the statistical test. Because the knowledge and attitude contingency tables had small expected cell counts, we estimated P values using a Monte Carlo exact procedure. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

3.4. Demographic and health-related associations

We assessed expected cell frequencies before selecting statistical tests. Fisher's exact test was used for monthly income because of a small expected cell count. Previous stool examination was significantly associated with preventive behavior ($P < 0.001$), with 90.2% of participants who had previously undergone stool

examination reporting adequate preventive behavior, compared with 74.6% of those who had never undergone examination. Marital status was also associated with preventive behavior ($P=0.04$). No statistically significant associations were observed for sex, age group, occupation, education level, monthly income, history of raw-food consumption, or underlying disease (Table 4).

Table 4. Associations between demographic and health-related characteristics and opisthorchiasis preventive behavior ($n=339$).

Variables	Suboptimal ($n=43$)	Adequate ($n=296$)	<i>P</i>
Sex			
Male	14 (11.5)	108 (88.5)	0.62
Female	29 (13.4)	188 (86.6)	
Age, years			
≤45	25 (14.0)	154 (86.0)	0.45
≥46	18 (11.3)	142 (88.8)	
Marital status			
Single	7 (6.9)	95 (93.1)	0.04
Married	36 (15.2)	201 (84.8)	
Occupation			
Farmer	26 (14.4)	155 (85.6)	0.32
Non-farmer	17 (10.8)	141 (89.2)	
Education			
Primary school	23 (12.7)	158 (87.3)	0.99
Higher than primary	20 (12.7)	138 (87.3)	
Monthly income, THB			
≤10000	39 (12.6)	271 (87.4)	0.77 [†]
≥10001	4 (13.8)	25 (86.2)	
History of raw-food consumption			
Ever	34 (12.4)	241 (87.6)	0.71
Never	9 (14.1)	55 (85.9)	
Previous stool examination			
Ever	27 (9.8)	249 (90.2)	<0.001
Never	16 (25.4)	47 (74.6)	
Underlying disease			
None	37 (14.0)	228 (86.0)	0.18
Present	6 (8.1)	68 (91.9)	

Footnote: Values are presented as n (%). P -values were obtained using Pearson's χ^2 -square test unless otherwise indicated. [†]Fisher's exact test was used because of a small expected cell count. We considered $P<0.05$ statistically significant.

3.5. Multivariable logistic regression analysis

The multivariable model included previous stool examination, knowledge score, and corrected attitude score because previous evidence has identified screening-related, knowledge, and attitudinal or behavioral factors as relevant to opisthorchiasis infection and preventive behavior[1,20-22]. Variable selection was based on

substantive and epidemiological relevance rather than solely on bivariate statistical significance, consistent with recommendations for multivariable model building[25]. All 339 participants included in the analysis had complete data. Preventive behavior was coded as 1 for adequate and 0 for suboptimal.

Adjusted analysis indicated that previous stool examination, knowledge score, and corrected attitude score were each independently associated with adequate preventive behavior. Individuals with a history of stool examination had approximately 2.8 times the adjusted odds of adequate preventive behavior compared with those without such a history (aOR 2.82, 95% CI 1.35-5.89, $P=0.006$). Each one-point increase in knowledge score corresponded to a 27% increase in the adjusted odds of adequate preventive behavior (aOR 1.27, 95% CI 1.13-1.43, $P<0.001$). Likewise, each one-point increase in corrected attitude score was associated with approximately 2.8 times the adjusted odds of adequate preventive behavior (aOR 2.76, 95% CI 1.51-5.02, $P=0.001$) (Table 5).

Model diagnostics indicated no evidence of problematic multicollinearity, as variance inflation factors ranged from 1.01 to 1.06. Box-Tidwell tests showed no evidence of departure from linearity in the logit for knowledge ($P=0.73$) or corrected attitude ($P=0.61$). The overall model was statistically significant [(likelihood-ratio $\chi^2(3)=33.28$, $P<0.001$)] and demonstrated adequate calibration [(Hosmer-Lemeshow $\chi^2(8)=8.36$, $P=0.40$)]. The model exhibited acceptable discrimination, with an area under the receiver operating characteristic curve of 0.776 (95% CI 0.703-0.848). Influence diagnostics showed a maximum Cook's distance of 0.062, with no observation approaching 1.

3.6. Sensitivity analysis

Analysis of the corrected preventive-behavior score as a continuous outcome yielded findings consistent with the primary binary logistic regression model. Previous stool examination, knowledge score, and corrected attitude score remained positively associated with preventive behavior (Supplementary Table 1). An additional ordinal analysis produced associations in the same direction; however, these findings should be interpreted cautiously because only one participant fell into the low preventive-behavior category, precluding reliable assessment of the proportional-odds assumption.

Table 5. Multivariable logistic regression analysis of factors associated with adequate preventive behavior ($n=339$).

Predictor	β	SE	aOR	95% CI	<i>P</i>
Previous stool examination: Ever vs. Never	1.037	0.376	2.82	1.35-5.89	0.006
Knowledge score	0.243	0.060	1.27	1.13-1.43	<0.001
Corrected attitude score	1.014	0.306	2.76	1.51-5.02	0.001
Constant (intercept)	6.220	1.599	-	-	<0.001

Footnote: Preventive behavior was coded as 1 for adequate and 0 for suboptimal. We included previous stool examination as a binary variable, with "Never" as the reference category. We analyzed knowledge and corrected attitude scores as continuous variables. aOR denotes adjusted odds ratio, CI indicates confidence interval, and SE represents standard error. All statistical tests were two-sided, and we considered P values <0.05 statistically significant.

4. Discussion

In this cross-sectional study, higher knowledge scores, more favorable attitudes toward opisthorchiasis prevention, and previous stool examination were independently associated with higher odds of adequate preventive behavior. These findings reflect associations rather than temporal or causal relationships. The high proportion of participants classified as having adequate preventive behavior also indicates limited outcome variability and warrants cautious interpretation of the observed associations.

Knowledge score was independently associated with preventive behavior. Each one-point increase in knowledge score was associated with a 27% increase in the adjusted odds of adequate preventive behavior (aOR 1.27, 95% CI 1.13–1.43). Knowledge may help people recognize infection risks and make informed decisions about preventive practices. Previous studies in northeastern Thailand have similarly demonstrated relationships between knowledge and opisthorchiasis-related preventive behaviors or infection risk[20–22,24]. Busabong *et al.* reported that knowledge, attitudes, and self-efficacy were relevant to *O. viverrini* and cholangiocarcinoma prevention in high-risk Thai communities[20]. Similarly, Maleelai *et al.* described knowledge and preventive behaviors among communities in Ubon Ratchathani Province[21]. Sornlorm *et al.* further demonstrated the importance of knowledge and behavioral factors in relation to *O. viverrini* infection in northeastern Thailand[22].

Nevertheless, the present findings should not be interpreted as evidence that greater knowledge causes improved preventive behavior. Given the cross-sectional design, the temporal relationship between knowledge and behavior cannot be established. Greater knowledge may have preceded preventive practices, but it may also have resulted from previous engagement with health services, screening activities, or other preventive experiences. Thus, knowledge should be considered one component within the broader context of opisthorchiasis prevention rather than an isolated determinant of behavior.

More favorable attitudes toward opisthorchiasis prevention were also independently associated with adequate preventive behavior (aOR 2.76, 95% CI 1.51–5.02). This finding is consistent with evidence suggesting that cognitive and attitudinal factors are relevant to preventive practices in communities at risk of *O. viverrini* infection[20,24]. However, favorable attitudes do not necessarily translate directly into preventive action. The intention-behavior gap indicates that favorable intentions or beliefs may not result in corresponding behavior when habitual, social, or environmental barriers remain[23]. Therefore, the observed association should not be interpreted as evidence that changing attitudes alone would produce sustained behavioral change.

The cultural and dietary context may be particularly important in interpreting these findings. Consumption of raw or inadequately

cooked freshwater fish remains central to *O. viverrini* transmission, and dietary practices are repeatedly identified as important risk factors for infection in endemic areas[1,3,4,12–14]. In the present study, more than four-fifths of participants reported a history of raw-food consumption, suggesting that exposure-related dietary practices remain relevant despite the generally favorable preventive-behavior scores. Previous research in northeastern Thailand has similarly demonstrated the continuing importance of food-related behaviors in *O. viverrini* infection and prevention[21,22,24].

However, in the present study, we measured raw-food consumption using only a broad self-reported ever/never history item. The questionnaire did not capture frequency, recency, specific fish species or dishes, preparation methods, portion size, or the social context of consumption. Consequently, the absence of a clear association between this broad exposure measure and preventive behavior should not be interpreted as evidence that dietary exposure is unimportant. Limited measurement of this variable may have led to exposure misclassification and residual confounding.

Previous stool examination was independently associated with adequate preventive behavior. Participants who had previously undergone stool examination had approximately 2.8 times the adjusted odds of adequate preventive behavior compared with those who had never undergone stool examination (aOR 2.82, 95% CI 1.35–5.89). Previous screening may represent greater engagement with health services, greater awareness of opisthorchiasis, previous exposure to health information, or participation in broader prevention activities. Integrated approaches to opisthorchiasis control have combined screening with health education and community-based prevention, rather than treating screening as an isolated behavioral intervention[2].

Nevertheless, the association observed in the present study should not be interpreted as evidence that stool examination itself improves subsequent preventive behavior. We did not collect information on the timing and frequency of previous examinations, test results, subsequent treatment, or screening-related health education. These factors could therefore not be distinguished from the observed association. Moreover, because we assessed previous stool examination and preventive behavior in a cross-sectional design, we could not establish their temporal sequence. Individuals already practicing more favorable preventive behaviors may have been more likely to participate in stool examination. Screening encounters may provide opportunities to reinforce preventive education, but whether integrating screening with health education produces subsequent and sustained behavioral change requires prospective evaluation.

The distribution of preventive-behavior scores also warrants consideration. Of the 339 participants, 296 (87.3%) were classified as having adequate preventive behavior and 43 (12.7%) as having suboptimal preventive behavior, indicating a pronounced concentration toward the favorable end of the scale. This ceiling

effect reduced variability in the primary binary outcome and may have limited the ability to detect some associations. Self-reported preventive behavior may also have been affected by recall and social desirability bias. Sensitivity analysis using the corrected preventive-behavior score as a continuous outcome produced associations in the same direction for previous stool examination, knowledge, and attitude, supporting the consistency of the primary findings. The ordinal sensitivity analysis also produced associations in the same direction but should be interpreted cautiously because only one participant was classified in the low preventive-behavior category.

Overall, the findings indicate that knowledge, attitudes, and previous stool examination were associated with preventive behavior in this endemic community. These findings support considering both cognitive factors and engagement with health services when developing opisthorchiasis prevention programs. Screening encounters may offer opportunities to reinforce preventive education; however, whether integrating screening and health education results in sustained behavioral change requires prospective or intervention studies.

From a public health perspective, the observed associations of knowledge, attitudes, and previous stool examination with preventive behavior suggest that opisthorchiasis prevention programs should consider both cognitive factors and opportunities arising from engagement with health services. Health education should aim not only to improve knowledge accuracy but also to promote favorable attitudes toward feasible preventive practices, particularly in communities where traditional dietary behaviors remain culturally embedded.

The association between previous stool examination and preventive behavior further suggests that screening encounters provide opportunities to reinforce culturally appropriate preventive education and counseling. However, the present findings do not establish that screening itself leads to behavioral change. Previous screening may instead reflect greater health awareness, prior preventive practices, exposure to health education, or greater engagement with health services. Future programs should prospectively evaluate whether integrating stool examination with targeted education and counseling improves and sustains preventive practices.

The present study also provides no direct evidence that improvements in knowledge, attitudes, or screening-related interventions reduce subsequent *O. viverrini* infection, cholangiocarcinoma risk, or long-term disease burden. Future longitudinal and intervention studies should therefore incorporate repeated behavioral assessments together with objective infection-related outcomes to determine whether changes in preventive behavior translate into reductions in infection and disease risk.

In conclusion, higher knowledge scores, more favorable attitudes toward opisthorchiasis prevention, and previous stool examination were associated with increased odds of adequate preventive behavior

among adults in this rural endemic community in northeastern Thailand. However, because this was a cross-sectional study, the temporal direction of these associations cannot be determined, and the results should not be interpreted as evidence that knowledge, attitudes, or previous screening directly cause behavioral improvement. Previous stool examination may also indicate broader engagement with health services or prior exposure to preventive information. These findings underscore the potential importance of addressing both cognitive factors and opportunities for preventive education during health-service encounters. Further research, particularly prospective longitudinal or intervention studies, is needed to determine whether integrating screening with culturally appropriate education and counseling improves and sustains preventive behavior or reduces *O. viverrini* infection and long-term disease burden.

This study has several limitations. First, detailed counts for intermediate recruitment stages were unavailable, preventing the calculation of stage-specific response rates and limiting the assessment of potential non-response bias. Nevertheless, the final analytical sample of 339 participants met the calculated sample-size requirement.

Second, the cross-sectional design precludes establishing temporality or causality. Reverse causation is possible, as participants with more favorable preventive behaviors may have been more likely to acquire health information or undergo stool examination. Self-reported measures may have introduced recall and social desirability bias. Additionally, the concentration of preventive-behavior scores at the upper end of the scale resulted in a ceiling effect, reducing outcome variability and potentially limiting our ability to detect certain associations.

Third, we measured previous stool examination and raw-food consumption using broad self-reported histories. We did not collect data on the timing or frequency of stool examinations, test results, subsequent treatment, screening-related health education, or detailed patterns of raw-food consumption. These limitations may have resulted in exposure misclassification and residual confounding. Furthermore, we did not comprehensively assess other potentially relevant factors, such as risk perception, social norms, household food practices, and access to preventive services.

Finally, eligibility criteria required participants to read and write Thai, which may have introduced selection bias by excluding individuals with lower literacy. Because the study was conducted in a single rural opisthorchiasis-endemic community in Ubon Ratchathani Province, the findings should be generalized with caution to populations with different literacy levels, dietary practices, sociocultural contexts, or access to health services.

The study setting and eligibility criteria limit the generalizability of these findings. Participants were recruited exclusively from a single rural opisthorchiasis-endemic community in Ubon Ratchathani

Province and were required to be literate in Thai. As a result, the findings may not apply directly to individuals with limited literacy, urban populations, or communities with different dietary practices, sociocultural contexts, or access to health services. However, these results offer valuable context-specific evidence regarding factors associated with preventive behavior in a rural opisthorchiasis-endemic community in northeastern Thailand.

Conflicts of interest statement

The authors declare no conflicts of interest.

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Availability of data

The datasets generated or analyzed during this study are available from the corresponding author upon reasonable request, in accordance with institutional ethical guidelines and participant confidentiality protections.

Declaration of use of generative AI and AI-assisted technologies

During the preparation and revision of this manuscript, the authors used ChatGPT (OpenAI) to assist with English-language editing, grammatical improvement, manuscript organization, and verification of the consistency of statistical reporting. The authors reviewed and edited all AI-assisted content and independently verified the statistical results against the study data. The authors take full responsibility for the manuscript's accuracy, integrity, interpretation, and final content.

Authors' contributions

KM contributed to the study's conceptualization and design. SB contributed to data acquisition. KM and SB contributed to data analysis and interpretation. KM drafted the initial manuscript and critically revised the manuscript. Both authors reviewed and approved the final version of the manuscript and agree to be accountable for the integrity of the work.

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Supplementary Table 1. Sensitivity analyses of factors associated with opisthorchiasis preventive behaviour ($n=339$).

Predictor	Estimate	95% <i>CI</i>	<i>P</i>
A. Linear regression using the corrected mean preventive-behaviour score			
Previous stool examination: Ever vs Never	$\beta=0.071$	0.008–0.134	0.027
Knowledge score	$\beta=0.0128$	0.004–0.021	0.003
Corrected attitude score	$\beta=0.0546$	0.015–0.094	0.006
B. Ordinal logistic regression using low, moderate, and high preventive-behaviour categories			
Previous stool examination: Ever vs. Never	<i>OR</i> =2.77	1.33–5.77	0.007
Knowledge score	<i>OR</i> =1.27	1.13–1.43	<0.001
Corrected attitude score	<i>OR</i> =2.73	1.51–4.96	0.001

Note: β , regression coefficient; *OR*, odds ratio; *CI*, confidence interval. Previous stool examination was coded as ever versus never, with never as the reference category. Knowledge and corrected attitude scores were entered as continuous variables. Model A used linear regression with heteroscedasticity-robust standard errors. Model B retained the ordered low, moderate, and high preventive-behaviour categories. Interpret the ordinal regression results cautiously because only one participant (0.3%) was classified in the low preventive-behaviour category. Because this category was so sparse, we could not reliably evaluate the proportional-odds assumption. Therefore, we treated the ordinal model as a supplementary sensitivity analysis rather than a primary inferential model.

RESEARCH QUESTIONNAIRE

Title: Questionnaire on factors associated with opisthorchiasis preventive behavior in a rural endemic community of northeastern Thailand: A cross-sectional study

Part 1: Sociodemographic Information

Instructions: Please place a ✓ in the appropriate box or fill in the information in the space provided.

1. Sex 1. Male 2. Female
2. Age: years (*Please indicate your completed age in years.*)
3. Marital Status 1. Single 2. Married 3. Divorced
4. Occupation
 1. Farmer
 2. Merchant/Business owner
 3. General worker
 4. Government officer
 5. Employee
 6. Other (please specify):
5. Educational Level
 1. Primary school
 2. Lower secondary school
 3. Upper secondary school
 4. Diploma/Associate degree
 5. Bachelor's degree
 6. Other (please specify):
6. Average Monthly Income: Thai Baht/month
7. History of Stool Examination for Liver Fluke Eggs
 1. Every year
 2. Never
 3. Occasionally
8. History of Eating Raw Food 1. No 2. Yes
9. Underlying Diseases/Chronic Conditions
 1. None
 2. Yes, please specify:

Part 2: Knowledge Assessment Questionnaire on Liver Fluke Infection

Instructions: Please place a ✓ in the box that best corresponds to your opinion. Select only one answer that you consider to be the most appropriate.

Yes 1 : means that you agree with the statement.

No 0 : means that you disagree with the statement.

Knowledge items on opisthorchiasis	Yes	No
<i>Causes</i>		
1. Adult liver flukes reside in the bile ducts.		
2. Opisthorchiasis is a major cause of cholangiocarcinoma.		
3. Opisthorchiasis is a major public health problem in Mekong basin countries including Thailand, Laos, Vietnam, and Cambodia.		
4. Opisthorchiasis can be completely cured simply by stopping consumption of raw fish.		
<i>Symptoms</i>		
5. Early symptoms of opisthorchiasis include abdominal bloating and epigastric discomfort.		
6. Key symptoms of opisthorchiasis include jaundice and yellowing of the eyes.		
7. Mild infection with liver flukes may not produce noticeable symptoms.		
8. Severe opisthorchiasis can cause marked abdominal bloating, occasional diarrhea, right upper quadrant pain, mild hepatomegaly, and low-grade fever.		
<i>Risky foods</i>		
9. Opisthorchiasis is transmitted through consumption of raw or undercooked cyprinid fish.		
10. Eating cooked fermented fish (<i>Pla-ra</i>) does not cause opisthorchiasis.		

11. Eating papaya salad containing raw fermented fish can transmit opisthorchiasis.		
12. Eating raw fermented fish dishes (<i>Pla-jom</i> , <i>Pla-som</i>) does not cause infection because fermentation kills the parasites.		
<i>Prevention</i>		
13. Fresh vegetables should be washed thoroughly before eating to prevent ingestion of parasite eggs.		
14. Adding lime juice to raw fish dishes (e.g., <i>Larb pla</i>) before eating can kill liver fluke parasites.		
15. Avoiding raw and undercooked fish cannot prevent opisthorchiasis.		
16. Handwashing before meals and after using the toilet can prevent contamination with liver fluke eggs.		
17. Stool examination is a diagnostic method for detecting liver fluke infection.		
18. Finding only a small number of liver fluke eggs in stool does not increase the risk of opisthorchiasis.		
<i>Treatment</i>		
19. Opisthorchiasis can be completely cured.		
20. The most effective treatment for opisthorchiasis is antiparasitic medication.		
21. Opisthorchiasis infection carries a high risk of death.		

Part 3: Attitudes Toward Liver Fluke Infection

Instructions: Please place a ✓ in the box that best reflects your opinion. Please select only one response for each statement.

Strongly Agree 5: means that you strongly agree with the statement., Agree 4: means that you agree with the statement., Neutral 3: means that you neither agree nor disagree with the statement., Disagree 2: means that you disagree with the statement., Strongly Disagree 1: means that you strongly disagree with the statement.

Item	Attitudes toward opisthorchiasis infection	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1	Squeezing lime and adding chili to raw freshwater fish before eating can kill liver fluke parasites.					
2	Food waste containing freshwater fish with white cysts should be discarded in tightly covered bins to prevent the spread of liver fluke disease.					
3	Eating <i>Somtam</i> with raw fermented fish (<i>Pla-ra</i>) can cause liver fluke infection.					
4	The best way to prevent liver fluke infection is taking deworming medication.					
5	Washing hands before meals and after using the toilet helps prevent the spread of liver fluke parasites.					
6	Cooking freshwater fish with white cysts takes too much time.					
7	Eating <i>Pla-som</i> (fermented fish) raw does not cause liver fluke infection because fermentation kills the parasites.					
8	Eating raw food is considered a form of social bonding.					
9	Not everyone who eats raw fish will develop liver fluke infection.					
10	Eating partially cooked (semi-raw) food strengthens the body.					
11	Making raw <i>Pla-ra</i> (fermented fish) at home and consuming it does not cause					

	liver fluke infection.					
12	Eating <i>Pla-ra</i> fermented for many years does not cause liver fluke infection because prolonged fermentation kills the parasites.					
13	Changing the habit of eating semi-raw foods is possible when necessary to prevent liver fluke infection.					

Part 4: Preventive Behaviors Against Liver Fluke Infection

Instructions: Please place a ✓ in the box that best reflects your usual behavior. Please select only one response for each statement.

Always 3 (6–7 days per week): means that you practice the behavior 6–7 days per week.

Sometimes 2 (3–4 days per week): means that you practice the behavior 3–4 days per week.

Never 1 (0 days per week): means that you do not practice the behavior at all.

Item	Opisthorchiasis prevention behaviors	Regularly	Sometimes	Never
1	Eat cooked freshwater fish with white cysts (e.g., silver barb, small cyprinid fish).			
2	Eat cooked <i>Naem pla</i> (fermented fish wrapped and grilled).			
3	Avoid eating partially cooked grilled fish (charred skin but reddish/raw flesh), as it may cause liver fluke disease.			
4	Eat raw fermented fish (<i>Pla-ra</i>) or raw <i>Jaew bong</i> (fermented fish chili paste).			
5	Wash fresh vegetables thoroughly before eating.			
6	Cook food thoroughly before eating, even if it reduces the taste.			
7	Wash your hands after handling raw fish before preparing other foods.			

8	Eat cooked fermented fish (<i>Pla-ra</i>) or cooked <i>Jaew bong</i> .			
9	Never buy foods made from raw fish.			
10	After cooking or eating, you dispose of food scraps or raw fish waste in a tightly covered bin.			
11	Wash cutting boards and knives after chopping fish with white cysts before using them to prepare other foods (e.g., vegetables, fruits).			
12	Wash your hands after defecation.			
13	Wash your hands before and after preparing and eating food.			
14	Attend stool examination services when available.			
15	Take antiparasitic medication.			