Prevalence and Associated Risk Factors of Intestinal Parasitic Infections in a Highland Community of Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand: A Two-Year Study

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Abstract

Intestinal parasitic infections remain a problem of public health in the tropical regions, particularly in rural communities with limited sanitation, poor hygiene practices, and environmental factors that facilitate parasite transmission. Despite the continuous implementation of national disease control programs, intestinal parasitic infections persist in certain geographically and culturally specific areas, including northeastern Thailand. However, the epidemiological data is relatively limited in highland rural areas specifically in Wang Nam Khiao District. This study aimed to investigate the two-year prevalence, analyze associated risk factors and confirm opisthorchiasis using molecular biology techniques in this area during 2024-2025. A repeated cross-sectional study was performed in 2024 (n = 183) and 2025 (n = 164), comprising a total of 347 fresh stool samples for simple direct smear, concentration techniques (FECT in 2024, EECT in 2025), and modified Agar Plate Culture (mAPC). PCR-based molecular identification was also carried out in 2025. Sociodemographic and behavioral risk factors were obtained through structured questionnaires. Potential risk factors were determined using logistic regression. The results showed that two-year prevalence of intestinal parasitic infection was 5.19%, decreasing from 6.01% in 2024 to 4.27% in 2025. Soil-transmitted helminths were most common parasites, with hookworm predominant in 2024 and *Strongyloides stercoralis* in 2025. Notably, *Opisthorchis viverrini*, a Group 1 carcinogen, was molecularly confirmed in 2025. Significant risk factors varied by year. Agricultural work was associated with infection in 2024, whereas daily alcohol consumption was a consistent risk factor and handwashing after touching pets or stray animals was protective in both years. In conclusion, this study provides valuable baseline data for intestinal parasitic infections and shows that these infections are still present as low levels in this rural highland community. The findings highlight show environmental factor and modifiable behaviors drive infection risk. Ongoing monitoring and targeted health education are key to improving local control.

Keywords: Intestinal parasitic infection, Prevalence, Risk factors, Repeated cross-sectional survey, Wang Nam Khiao District

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ความชุกและปัจจัยเสี่ยงที่เกี่ยวข้องกับการติดเชื้อปรสิตในลำไส้ในชุมชนพื้นที่สูง อำเภอวังน้ำเขียว จังหวัดนครราชสีมา ประเทศไทย: การศึกษา 2 ปีต่อเนื่อง

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บทคัดย่อ

การติดเชื้อปรสิตในลำไส้ยังคงเป็นปัญหาด้านสาธารณสุขในเขตร้อน โดยเฉพาะในชุมชนชนบทที่ยังมีข้อจำกัดด้านสุขาภิบาลและพฤติกรรมสุขอนามัยรวมถึงปัจจัยด้านสิ่งแวดล้อมที่เอื้อต่อการแพร่กระจายของเชื้อ แม้จะมีการดำเนินโครงการควบคุมโรคระดับชาติอย่างต่อเนื่องแต่การติดเชื้อยังคงพบได้ในบางพื้นที่ที่มีบริบทเฉพาะด้านภูมิประเทศและวิถีชีวิตของประชาชน รวมถึงในภาคตะวันออกเฉียงเหนือของประเทศไทย อย่างไรก็ตามข้อมูลระบาดวิทยาในพื้นที่ชนบทบนที่สูงโดยเฉพาะอำเภอวังน้ำเขียวยังมีจำกัด การศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาความชุกในช่วงระยะเวลา 2 ปี วิเคราะห์ปัจจัยเสี่ยงที่เกี่ยวข้อง และยืนยันการติดเชื้อพยาธิใบไม้ตับด้วยเทคนิคอณูชีววิทยา ในพื้นที่ดังกล่าว ช่วงปี พ.ศ. 2567-2568 วิธีการศึกษาดำเนินแบบภาคตัดขวางซ้ำ โดยเก็บตัวอย่างอุจจาระสดทั้งหมด 347 ตัวอย่าง จากกลุ่มตัวอย่าง 183 รายในปี พ.ศ. 2567 และ 164 รายในปี พ.ศ. 2568 ศึกษาด้วยวิธีตรวจโดยตรงอย่างง่าย วิธีตรวจโดยทำให้เข้มข้น (FECT ในปี พ.ศ. 2567 และ EECT ในปี พ.ศ. 2568) วิธีเพาะเลี้ยงบนอาหาร และตรวจยืนยันชนิดของเชื้อด้วยวิธี PCR ในปี พ.ศ. 2568 ร่วมกับการสำรวจข้อมูลปัจจัยเสี่ยง โดยใช้แบบสอบถาม และการวิเคราะห์ถดถอยโลจิสติกส์ ผลการศึกษาความชุกของการติดเชื้อปรสิตในลำไส้ระยะเวลา 2 ปี พบร้อยละ 5.19 โดยลดลงจากร้อยละ 6.01 ในปี พ.ศ. 2567 เป็นร้อยละ 4.27 ในปี พ.ศ. 2568 โดยปรสิตกลุ่มที่ติดต่อผ่านดินเป็นชนิดที่พบส่วนใหญ่ ได้แก่ พยาธิปากขอ ในปี พ.ศ. 2567 และพยาธิสตรองจิรอยด์ ในปี พ.ศ. 2568 นอกจากนี้ ยังตรวจพบพยาธิใบไม้ในตับ ซึ่งจัดเป็นสารก่อมะเร็งกลุ่มที่ 1 ด้วยเทคนิคอณูชีววิทยาในปี พ.ศ. 2568 สำหรับปัจจัยเสี่ยงที่มีนัยสำคัญแตกต่างกันในแต่ละปี โดยอาชีพเกษตรกรรมสัมพันธ์กับการติดเชื้อในปี พ.ศ. 2567 ขณะที่การดื่มแอลกอฮอล์เป็นประจำเป็นปัจจัยเสี่ยงที่พบอย่างสม่ำเสมอ และการล้างมือหลังสัมผัสสัตว์มีความสัมพันธ์กับการลดความเสี่ยงของการติดเชื้อในทั้งสองปี สรุป การศึกษานี้ให้ข้อมูลพื้นฐานที่มีคุณค่าต่อสถานการณ์การติดเชื้อปรสิตในลำไส้ที่ยังคงพบในระดับต่ำในชุมชนชนบทบนที่สูง ปัจจัยด้านสิ่งแวดล้อมและพฤติกรรมเสี่ยงเป็นตัวขับเคลื่อนหลัก การเฝ้าระวังอย่างต่อเนื่องและการให้สุขศึกษา จึงเป็นกุญแจสำคัญในการยกระดับการควบคุมโรคในท้องถิ่น

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1. Introduction

Intestinal parasitic infections caused by protozoa and helminths remain a neglected tropical disease, which is major public health concern worldwide affecting approximately 1.5 billion people, particularly in tropical and subtropical regions.[1] Clinical manifestations of these parasites range from mild symptoms to severe disease, and chronic infections can lead to significant morbidity and mortality, especially among immunocompromised hosts.[2]

Thailand, located in the tropical Greater Mekong Subregion of Southeast Asia, continues to report intestinal parasitic cases with regional variation. Although the Ministry of Public Health, through the Department of Disease Control has implemented nationwide programs to promote preventive measures against parasitic infections among the Thai population. This has led to a decreasing trend in the prevalence of parasitic infections since 2002 (according to the Department of General Communicable Diseases, Department of Disease Control, Ministry of Public Health, 2018). However, infection rates vary due to geography, climate variations, sanitation, occupation and lifestyle behaviors.[3] Understanding local prevalence and associated risk factors is crucial for targeted interventions to reduce morbidity, hospitalization, and mortality, particularly in communities lacking baseline epidemiological data. In Thailand, helminth infections are more prevalent than protozoa infection with the two mains parasite groups are nematodes, especially soil-transmitted helminths (STHs) and Platyhelminthes.[4] Soil-transmitted helminths (3.1-15.7%) especially hookworm and *Strongyloides stercoralis* are still a chronic issue affecting public health significantly in Thailand.[4,5] *S. stercoralis* cause lifelong autoinfection and severe hyperinfection, particularly among immunocompromised hosts.[6,7] Transmission is driven by consumption of contaminated vegetable, fruit or water contaminated, poor hygiene practices, poor sanitation, and exposure to contaminated soil or water and also climatic conditions such as high humidity and warm temperatures enhance parasite survival and transmission, increasing public health risk.[5,8] Notably, *Opisthorchis viverrini*, a Group I carcinogen associated with cholangiocarcinoma (CCA), shows varying prevalence level in northeastern Thailand, ranging from less than 5% to more than 40% in low- and high-endemic communities, respectively.[9,10] Humans commonly acquire *O. viverrini* through consumption raw, fermented or undercooks of cyprinid fish.[11] In addition, common protozoa include *Blastocystis* spp., *Entamoeba coli*, *Entamoeba histolytica*, and *Giardia duodenalis.*[4,5] These findings reveal the uneven distribution of parasitic infections across Thailand and emphasize the need for targeted local epidemiological studies.

Nakhon Ratchasima is second-largest province of Thailand by area, following Chiang Mai, and largest province in northeastern Thailand. The Wang Nam Khiao District is located in the southern highland area of this province, characterized by cool, humid climate, dense forest cover and mountainous terrain. Most residents are engaged in agriculture, resulting in frequent exposure to soil and natural water that play key pathways for the transmission of parasite.[12] While ecotourism brings tourists and temporary workers may contribute to pathogen importation and spread.[13] Despite environmental conditions may support parasite transmission, the prevalence and risk factors of intestinal parasitic infections in Wang Nam Khiao District remain poorly documented. This is critical knowledge gap, as understanding local epidemiology plays an important role in design of the most effective community-based control programs. Baseline data is essential where ecological and behavioral factors differ from other regions.

The objective of this study was to investigate the two-year prevalence of intestinal parasitic infections, risk factors and confirmation of Opisthorchiasis by molecular method in Thai Samakkhi Subdistrict, Wang

Nam Khiao District during 2024-2025. The findings provide valuable information about local transmission dynamics as well as inform future surveillance and parasite control measure for rural highland area.

2. Materials and Methods

2.1 Study design and setting

Repeated cross-sectional survey over two consecutive years was performed in highland area in Northeastern Thailand (May 2024 and May 2025) Thai Samakkhi Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province. It is a rural area located at latitude 14.358610°N and longitude 101.921965°E, about 235 km northeast of Bangkok, Thailand. This subdistrict covers an area of approximately 315 km^2, comprising 11 administrative villages with a total population of 7,208 as of 2023 (The Official Statistics Registration Systems, Thailand). The geography of Wang Nam Khiao district is characterized by high mountains and rolling hills with a cool, foggy, and humid climate throughout the year. The majority of residents are involved in agriculture such as growing rice and sugarcane, cassava farming, and fruit and vegetable gardening.

2.2 Study population and sample size

The sample size was determined using the following statistic formula:

$$n = \frac{NZ^2\,P(1-P)}{d^2(N-1)+Z^2\,P(1-P)} = \frac{7208 * 1.96^2 * 0.0505(1-0.0505)}{0.05^2(7208-1)+1.96^2\,0.0505(1-0.0505)} = 72.95 \approx 73$$

When, n is the required sample size, N is population size (7208), Z is the Z-score for 95% confidence interval, d is the acceptable error (5%), P is the expected prevalence (5.05%).[14] Thus, the minimum sample size for the calculation was 73 individuals.

The inclusion criteria included individuals residing in Thai Samakkhi Subdistrict of Wang Nam Khiao District, Nakhon Ratchasima Province and they provided the informed consent of ethics document. The exclusion criteria included participants who had taken antiparasitic drugs within the past 6 months and those who did not submit the questionnaire.

2.3 Sample collection and parasitological survey

A single fresh stool sample was collected from 183 participants in May 2024 and 164 participants in May 2025, totaling 347 fresh stool samples. Approximately 5-10 g of each stool samples was collected using a clean stool container and immediately transported to parasitology laboratory at Nakhonratchasima college using ice pack (around 4-10°C). Three diagnostic methods were used to examine for intestinal parasites infection from fresh stool samples as follow; the first was simple direct smear method both in 0.85% normal saline and 1% iodine solution and observed under a light microscope. Subsequently, the modified Agar Plate Culture (mAPC) technique was performed to detect the larval stage of *Strongyloides stercoralis* and Hookworm. Briefly, 2 g of fresh stool sample were plated on nutrient agar plate with pH 6 and incubated at 30°C for 3-7 days and then being observed under a stereomicroscope.[15]

Stool concentration methods were performed in both survey years to enhance the detection of egg and the larval stage of intestinal parasite. In 2024, the formalin ethyl acetate concentration technique (FECT) was used, which approximately 2 g of stool was mixed with 10% formalin as the preservative as previous described.[16] In 2025, an updated protocol, the ethanol ethyl acetate concentration technique (EECT), was applied using 80% ethanol in place of formalin. Both methods followed similar procedural steps, Briefly, 2 g of fresh stool sample was mixed with preservative solution and filtered it through two layers of sterile

gauze into sterile centrifuge tube. The preservative solution was added to adjusted as 10 ml and centrifuged at 1500 rpm for 2 minutes. The supernatant was discarded and repeated this around 2-3 times. Then, 7 ml of preservative solution were added and leave it for 5 minutes, follow by mixed it well with 3 ml of ethyl acetate and centrifuged at 1500 rpm for 5 minutes. The sediment was investigated under a light microscope.

A double-blind design was used for the parasitological examination. All samples were independently examined in duplicate by the same two experienced medical technologists across both study years, following standardized protocols.

2.4 Genomic DNA isolation

Genomic DNA was isolated from sediments obtained through the EECT procedure that contained *O. viverrini*-like eggs. For *O. viverrini*, the sediment was first processed to autoclaving at 121°C for 5 minutes to open the operculum and facilitate release of internal material prior to DNA extraction.[14, 17] Genomic DNA was then extracted using the TIANamp Stool DNA Kit (TIANGEN®, China), following the manufacturer's protocol. The final elution volume for each sample ranged between 50 µL.

2.5 Molecular identification

Because the eggs of *Opisthorchis viverrini* (Ov) and minute intestinal flukes (MIFs) share similar morphological characteristics, molecular confirmation of *O. viverrini* was performed using specific PCR amplification targeting the OvNad5 gene, as previously described.[14] The PCR reaction generated a 199 bp amplicon using 25 pmol of each primer (OvNad5-F: 5'-TTT GCG GAG GTT TGT TAC CT-3'; OvNad5-R: 5'-CAC CTC ACC AAT TCA ACA CG-3') and 3 µL of genomic DNA. PCR amplification was conducted using a PR-96E Gradient PCR instrument (Hangzhou Miu, China) with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing 55°C for 1 minute, and extension 72°C for 1 minute, and a final extension at 72°C for 10 minutes. Amplicons from both assays were separated on 1.5% agarose gel containing ViSafe Red Gel Stain (Vivantis, USA) using 1× TBE buffer at 70 V for 40 minutes. Genomic DNA from adult *O. viverrini* served as the positive control and sterile distilled water was used as the negative control.

2.6 Questionnaire survey

All participants were collected questionnaire that consist three domains. The first domain included sociodemographic data including gender, age, marital status, occupation and education level. The second domain collected clinical information including any medical history, taken antiparasitic drugs. The third domain assessed behavioral data associated with intestinal parasitic infection including dietary habits (e.g., consumption of raw/ undercooked food and fresh vegetables), hygiene practices (e.g., washing fresh vegetables/ fruits, handwashing, wearing shoes), and lifestyle behaviors (e.g., smoking and alcohol consumption). The same questionnaire was used in both survey years (2024 and 2025) to ensure consistency and comparability of data across the two study periods.

2.7 Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethical Committee of Nakhonratchasima College (NMCEC-0024/2566). All participants provided the informed consent in both survey years (2024 and 2025). Positive cases were referred to the Ban Thai Samakkhi Health Center, Wang Nam Khiao District, Nakhon Ratchasima Province for appropriate treatment.

2.8 Statistic analysis

Statistical analyses were performed using IBM SPSS Statistics (Version 26). Prevalence of intestinal parasitic infection was described using percentage. Relationship between health behaviors and parasitic infections using the Pearson Chi-square test or Fisher's exact test (when 20% or more of the expected counts were less than 5). Binary logistic regression was used to analyze factors associated with intestinal parasitic infection. *p*-values of less than 0.05 were considered to be statistically significant at a 95% CI. Percentages for descriptive variables were calculated from available data, which may result in totals deviating from 100% due to incomplete responses.

3. Results

3.1 Sociodemographic characteristics

Participants were recruited from all 11 villages of Thai Samakkhi subdistrict to ensure comprehensive geographic coverage and to minimize potential sampling bias. A total of 183 participants in 2024 were enrolled comprising 118 females (64.48%, positive cases 5.08%) and 65 males (35.52%, positive cases 7.69%). The average age was 50.08 ± 15.65 years, with most participants age between 40-59 years and the highest infection observed in those aged ≥ 60 years (9.43%). Freelance workers constituted the largest occupational group (46.99%), whereas agricultural workers showed a significantly higher proportion of parasitic infections (25.14%, positive cases 13.04%, $p = 0.031$). Abdominal bloating and distension symptoms were also significantly correlated with infection (20.22%, positive cases 13.51%, $p = 0.026$) as shown in Table 1. Other demographic factors and underlying diseases were not significant associated with parasitic infection.

In 2025, 164 subjects were recruited including 107 females (65.24%, positive cases 2.80%) and 57 males (34.76%, positive cases 7.02%) with a mean age being 56.57 ± 12.42 years and the highest infection observed in those aged ≥ 60 years (6.00%). Demographic patterns in 2025 were similar to those in 2024. Abdominal bloating and distension were the only symptom significantly linked to infection ($p = 0.011$). No sociodemographic factors showed statistically significant association with infection.

Several behavioral risk factors were consistently associated with parasitic infection across both years. Daily alcohol consumption showed a strong association with parasitic infection ($p = 0.039$ in 2024; $p = 0.005$ in 2025) with heavier drinkers at highest risk. Proper handwashing after contact with pets or stray animals was also significantly associated with a reduced risk of parasitic infection in both years ($p = 0.017$ in 2024; $p = 0.046$ in 2025), highlighting the importance of hygiene in preventing zoonotic exposure. Additionally, Smoking and consumption of raw or undercooked food were a significantly related with parasitic infection only in 2025 ($p = 0.000$ and $p = 0.049$, respectively), whereas no significant associations were observed in 2024. Other hygiene practices and lifestyle factors such as washing vegetables before consumption, handwashing before eating, handwashing after using toilet, the use of safe drinking water source and sanitary toilet excreta disposal method showed no significant association with parasitic infection as presented in Table 1.

Table 1 Sociodemographic data and health behavior factors associated with intestinal parasitic infections among participants from Thai Samakkhi communities of Wang Nam Khiao District, Nakhon Ratchasima Province during 2024 and 2025.

Demographic characteristic	2024, n (%)			2025, n (%)		
	Total	Parasitic infection	*p*-value	Total	Parasitic infection	*p*-value
Total	**183**	**11 (6.01)**		**164**	**7 (4.27)**	
Gender			0.524[b]			0.238[b]
Female	118 (64.48)	6 (5.08)		107 (65.24)	3 (2.80)	
Male	65 (35.52)	5 (7.69)		57 (34.76)	4 (7.02)	
Age (Years)			0.679[a]			0.404[a]
Mean age (Mean ± SD)	50.08 ± 15.65			56.57 ± 12.42		
≤ 39	40 (21.86)	1 (2.5)		31 (18.90)	0 (0.00)	
40-59	89 (48.63)	5 (5.62)		83 (50.61)	4 (4.82)	
≥ 60	53 (28.96)	5 (9.43)		50 (30.49)	3 (6.00)	
Marital status			0.746[a]			0.921[a]
Single	32 (17.49)	1 (3.13)		22 (13.41)	1 (4.55)	
Married	109 (59.56)	7 (6.42)		104 (63.41)	5 (4.81)	
Divorced	23 (12.57)	1 (4.35)		32 (19.51)	1 (3.13)	
Education			0.687[a]			0.477[a]
Primary school	88 (48.09)	5 (5.68)		71 (43.29)	5 (7.04)	
Secondary school	85 (46.45)	5 (5.88)		75 (45.73)	2 (2.67)	
Bachelor degree	4 (2.19)	0 (0.00)		10 (6.10)	0 (0.00)	
Not enrolled	6 (3.28)	1 (16.67)		6 (3.66)	0 (0.00)	
Occupation						
Freelance	86 (46.99)	5 (5.81)	1.000[b]	60 (36.59)	4 (6.67)	0.261[b]
Agriculture	46 (25.14)	6 (13.04)	0.031[b*]	45 (27.44)	2 (4.44)	1.000[b]
Seller/ merchant	23 (12.57)	0 (0.00)	0.364[b]	26 (15.85)	2 (7.69)	0.307[b]
Student	7 (3.83)	0 (0.00)	1.000[b]	1 (0.61)	0 (0.00)	1.000[b]
Government officer	3 (1.64)	0 (0.00)	1.000[b]	5 (3.05)	0 (0.00)	1.000[b]
Unemployed	19 (10.38)	1 (5.26)	1.000[b]	19 (11.59)	2 (10.53)	0.188[b]
Underlying disease						
Diabetes	26 (14.21)	4 (15.38)	0.075[b]	26 (15.85)	2 (7.69)	0.307[b]
Hypertension	45 (24.59)	6 (13.33)	0.072[b]	44 (26.83)	2 (4.55)	1.000[b]
Obesity	4 (2.19)	0 (0.00)	1.000[b]	5 (3.05)	0 (0.00)	1.000[b]
Kidney disease	2 (1.09)	0 (0.00)	1.000[b]	5 (3.05)	0 (0.00)	1.000[b]
Symptoms						
Abdominal pain	15 (8.20)	0 (0.00)	1.000[b]	19 (11.59)	2 (10.53)	0.188[b]
Abdominal bloating and distension	37 (20.22)	5 (13.51)	0.026[b*]	41 (25.00)	5 (12.20)	0.011[b*]
Red, itchy rash on the skin	10 (5.46)	0 (0.00)	1.000[b]	11 (6.71)	0 (0.00)	1.000[b]
Rapid weight loss	3 (1.64)	0 (0.00)	1.000[b]	2 (1.22)	0 (0.00)	1.000[b]
Smoking			0.457[a]			0.000[a*]
Daily smoker	28 (15.30)	1 (3.57)		20 (12.20)	1 (5.00)	
Frequent smoker	2 (1.09)	0 (0.00)		1 (0.61)	1 (100.00)	
Occasional smoker	0 (0.00)	0 (0.00)		1 (0.61)	0 (0.00)	
Ex-smoker	22 (12.02)	3 (13.64)		15 (9.15)	1 (6.67)	
Never	124 (67.76)	7 (5.65)		120 (73.17)	4 (3.33)	
Alcohol consumption			0.039[a*]			0.005[a*]
Daily drinker	14 (7.65)	3 (21.43)		12 (7.32)	3 (25.00)	
Frequent drinker	15 (8.20)	0 (0.00)		18 (10.98)	1 (5.56)	
Occasional drinker	35 (19.13)	1 (2.86)		30 (18.29)	2 (6.67)	
Ex-drinker	22 (12.02)	3 (13.64)		14 (8.54)	0 (0.00)	
Never	94 (51.37)	4 (4.26)		83 (50.61)	1 (1.20)	

Table 1 Sociodemographic data and health behavior factors associated with intestinal parasitic infections among participants from Thai Samakkhi communities of Wang Nam Khiao District, Nakhon Ratchasima Province during 2024 and 2025. (Cont.)

Demographic characteristic	2024, n (%)			2025, n (%)		
	Total	Parasitic infection	*p*-value	Total	Parasitic infection	*p*-value
Habit of consuming raw or undercooked food			0.631[b]			0.049[b]*
Yes	22 (12.02)	2 (9.09)		70 (42.68)	6 (8.57)	
No	154 (84.15)	9 (5.84)		82 (50.00)	1 (1.22)	
Habit of consuming fresh vegetables			1.000[b]			1.000[b]
Yes	169 (92.35)	11 (6.51)		145 (88.41)	7 (4.83)	
No	9 (4.92)	0 (0.00)		6 (3.66)	0 (0.00)	
Walking barefoot on soil			0.751[b]			0.699[b]
Yes	115 (62.84)	8 (6.96)		86 (52.44)	5 (5.81)	
No	60 (32.79)	3 (5.00)		63 (38.41)	2 (3.17)	
Washing vegetable or fruit before consumption			1.000[b]			1.000[b]
Yes	169 (92.35)	11 (6.51)		147 (89.63)	7 (4.76)	
No	4 (2.19)	0 (0.00)		3 (1.83)	0 (0.00)	
Handwashing before eating			1.000[b]			1.000[b]
Yes	172 (93.99)	11 (6.40)		149 (90.85)	7 (4.70)	
No	4 (2.19)	0 (0.00)		2 (1.22)	0 (0.00)	
Handwashing after using toilet			0.517[b]			0.134[b]
Yes	166 (90.71)	10 (6.02)		147 (89.63)	6 (4.08)	
No	11 (6.01)	1 (9.09)		3 (1.83)	1 (33.33)	
Handwashing after touch pets or stray animals			0.017[b]*			0.046[b]*
Yes	167 (91.26)	8 (4.79)		142 (86.59)	5 (3.52)	
No	10 (5.46)	3 (30.00)		8 (4.88)	2 (25.00)	
Safe drinking water source			0.384[b]			0.564[b]
Safe	28 (15.30)	3 (10.71)		18 (10.98)	1 (5.56)	
Unsafe	149 (81.42)	8 (5.37)		146 (89.02)	6 (4.11)	
Toilet excreta disposal method			0.602[b]			0.595[b]
Sanitary	162 (88.52)	11 (6.79)		125 (76.22)	6 (4.80)	
Unsanitary	15 (8.20)	0 (0.000)		29 (17.68)	0 (0.00)	

* *P*-value < 0.05 using a = Pearson Chi-square test, b = Fisher's Exact test, SD = Standard Deviation

3.2 Prevalence of intestinal parasitic infections

A total of 347 fresh stool specimens were collected from community residents over a two-year period (183 samples in 2024 and 164 samples in 2025). Overall prevalence of intestinal parasitic infections was 5.19% (18/347), with annual prevalence rates of 6.01% (11/183) in 2024 and 4.27% (7/164) in 2025 (Table 2).

Protozoan infections were detected at low prevalence in both years. In 2024, we found 4 cases (2.19%) including *Iodamoeba butschlii* (0.55%), coinfections of *Blastocystis* spp. and *Entamoeba coli* (1.09%), together with coinfection of *Giardia duodenalis* and *Iodamoeba butschlii* (0.55%). In 2025, three protozoan infections (1.83%) were detected including *Blastocystis* spp. (0.61%), *Entamoeba histolytica/Entamoeba*

dispar (0.61%), and coinfection of *Pentatrichomonas hominis* and *Blastocystis* spp. (0.61%). Overall, protozoan infections found 2.02% of all sample across both years.

Soil transmitted helminths dominated in this community with hookworms at 3.27% (6/183) in 2024 and 0.61% (1/164) in 2025, totaling 2.02% (7/347). As well as *Strongyloides stercoralis* was detected once in 2024 (0.55%) and twice in 2025 (1.22%), resulting in an overall prevalence of 0.86% (3/347). Notably, one case of *Opisthorchis viverrini* was identified in 2025 (0.61%) and confirmed by PCR method, as evidenced by a specific DNA band at 199 bp (Fig. 2). This is illustrated in Table 2 and representation images of the detected parasites shown in Fig. 1 and 2.

Table 2 Prevalence and distribution of intestinal parasitic infections in fresh stool sample from participants (2024-2025).

Type of Intestinal parasitic Infection	Year, n (%)		
	2024 (n = 183)	2025 (n = 164)	Total (n = 347)
Intestinal protozoa (total)	**4 (2.19)**	**3 (1.83)**	**7 (2.02)**
Single infection			
Iodamoeba butschlii	1 (0.55)	0	1 (0.29)
Blastocystis spp.	0	1 (0.61)	1 (0.29)
Entamoeba histolytica/ Entamoeba dispar	0	1 (0.61)	1 (0.29)
Coinfection			
Blastocystis spp. + *Entamoeba coli*	2 (1.09)	0	2 (0.57)
Giardia duodenalis + *Iodamoeba butschlii*	1 (0.55)	0	1 (0.29)
Pentatrichomonas hominis + *Blastocystis* spp.	0	1 (0.61)	1 (0.29)
Intestinal helminths (total)	**7 (3.82)**	**4 (2.44)**	**11 (3.17)**
Single infection			
Opisthorchis viverrini	0	1 (0.61)	1 (0.29)
Hookworm	6 (3.27)	1 (0.61)	7 (2.02)
Strongyloides sterocoralis	1 (0.55)	2 (1.22)	3 (0.86)
Overall prevalence	**11 (6.01)**	**7 (4.27)**	**18 (5.19)**

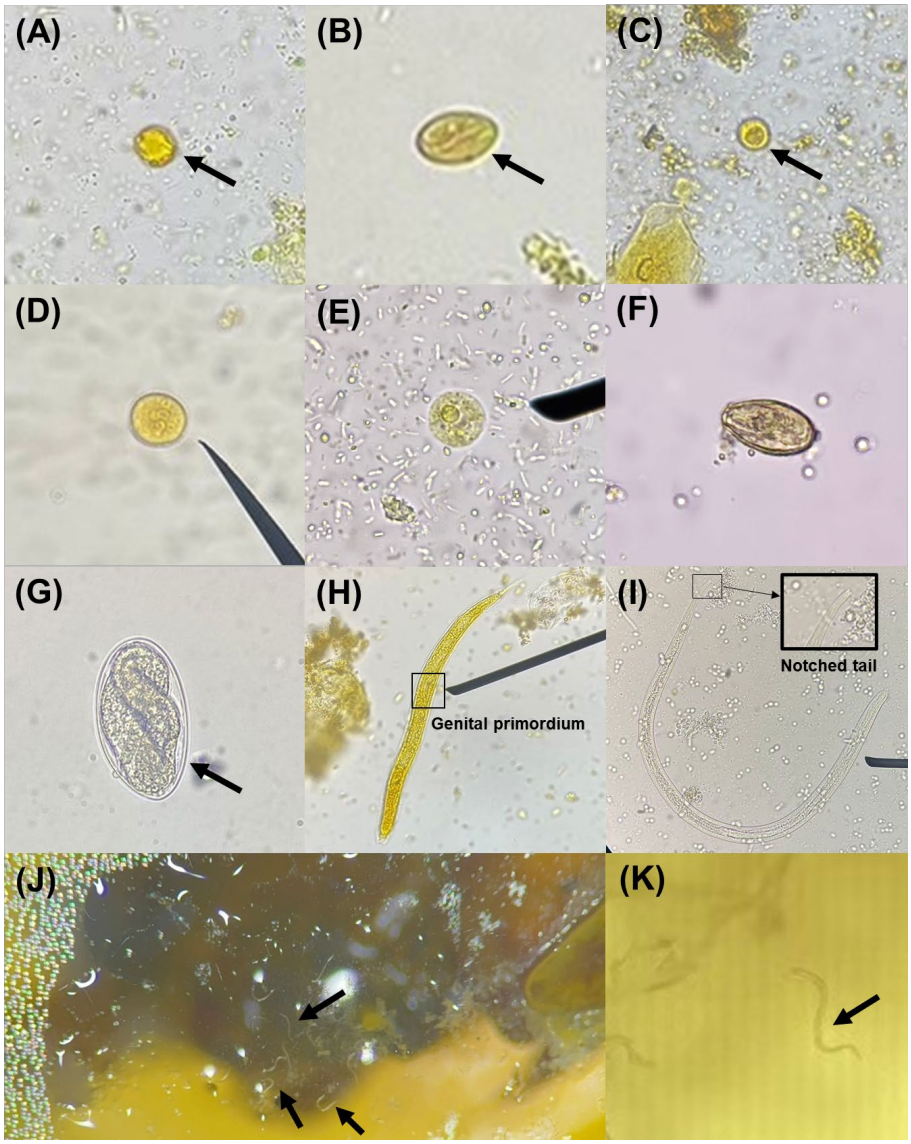


Fig. 1 Representative images of parasite eggs and larva were detected by the simple direct smear, concentration technique (Formalin-ethyl acetate concentration technique, FECT in 2024; Ethanol-ethyl acetate concentration technique, EECT in 2025) and modified Agar plate culture (mAPC) at 400X magnification. (A) *Blastocystis* spp. cyst in 1% iodine of simple direct smear, (B) *Giardia duodenalis* cyst in 1% iodine of simple direct smear, (C) *Iodamoeba butschlii* in 1% iodine of simple direct smear, (D) *Entamoeba coli* cyst in 1% iodine of simple direct smear, (E) *Entamoeba histolytica/Entamoeba dispar* cyst in 1% iodine of simple direct smear, (F) *Opisthorchis viverrini* egg in 0.85% normal saline of 80% EECT, (G) Hookworm embryonated egg in 0.85% Normal saline of FECT, (H) *Strongyloides stercoralis* rhabditiform larva in 1% iodine of FECT, (I) *Strongyloides stercoralis* filariform larvae in 0.85% Normal saline of mAPC, (J, K) Positive case identified in the mAPC using stereomicroscope.

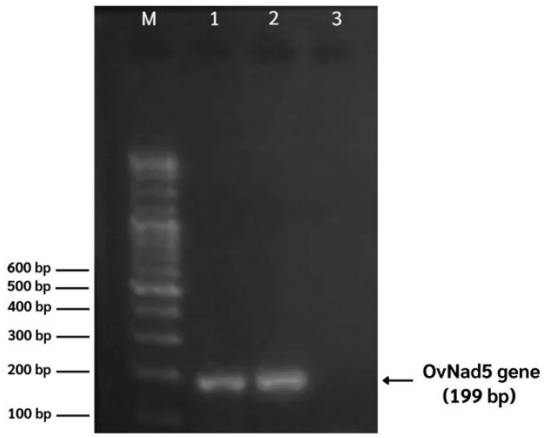


Fig. 2 PCR amplification of *Opisthorchis viverrini* targeting the OvNad5 gene (199 bp), visualized by 1.5% agarose gel electrophoresis. Lane M: 100-3000 bp DNA ladder, Lane 1: DNA from *O. viverrini*-like eggs (EECT stool sample), Lane 2: *O. viverrini* positive control, Lane 3: negative control. The presence of a distinct 199 bp band (arrow) confirms the detection of *O. viverrini*.

3.3 Potential risk factors for intestinal parasitic infection

The results of binary logistic regression analyses identifying potential risk factors for intestinal parasitic infections are presented in Table 3. For result in 2024, agricultural occupation revealed a significant related with parasitic infection in the univariable model (COR = 3.96; 95%, CI: 1.148-13.663, p = 0.029). After adjustments with gender, age, agricultural exposure, alcohol consumption and handwashing after touching pets or stray animals found that agricultural work still a significant predictor (AOR = 7.922, 95% CI: 1.672-37.535, p = 0.009), indicating an increased risk among individuals engaged in farm-related activities. Daily drinkers showed a markedly increased risk in both analyses (COR = 6.136, 95% CI: 1.211-31.088, p = 0.028) and (AOR = 8.384; 95%, CI: 1.061-66.233, p = 0.044). Daily alcohol consumption also predominant as a significant behavioral factor. In addition, proper handwashing after touching pets or stray animals was significantly associated with a reduced risk of infection (COR = 0.117, 95% CI: 0.025-0.541; p = 0.006), and this protective effect remained significant after adjustment (AOR = 0.127, 95% CI: 0.018-0.878, p = 0.036).

In 2025, demographic data including gender, age, marital status, and education showed no significant related to infection. Daily alcohol consumption remained a strong and consistent predictor of infection. Daily drinkers had significantly higher odds of infection in both univariable (COR = 27.333, 95% CI: 2.567-291.084, p = 0.006) and multivariable models (AOR = 26.990, 95% CI: 1.492-488.156, p = 0.026) when adjustment data with gender, age, alcohol consumption, handwashing after using toilet and handwashing after touching pets or stray animals, indicating a persistent behavioral risk pattern across study years. Handwashing behavior after touching pets or stray animals was also significantly associated in crude analysis (COR = 0.109, 95% CI: 0.018-0.684, p = 0.018). Although the association lost statistical significance after adjustment (AOR = 0.420, 95% CI: 0.020-8.610, p = 0.574), the direction of effect still suggested a protective role of proper hygiene. Other behavioral factors including consumption of raw foods, walking barefoot on soil, handwashing before eating, handwashing after using toilet, water source safety was significantly associated with parasitic infection in either analysis both years.

Table 3 Univariable and multivariable logistic regression analysis of factors associated with intestinal parasitic infections among participants in 2024 and 2025.

Variables	2024				2025			
	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
Gender								
Male	1.556 (0.456-5.309)	0.481	1.602 (0.372-6.896)	0.527	2.616 (0.565-12.120)	0.219	0.864 (0.123-6.063)	0.883
Female	1		1		1		1	
Age								
≤ 39	0.246 (0.028-2.196)	0.209	0.262 (0.023-2.992)	0.281	0.000 (0.000)	0.998	0.000 (0.000)	0.998
40-59	0.571 (0.157-2.074)	0.395	0.553 (0.127-2.411)	0.430	0.793 (0.170-3.700)	0.768	0.309 (0.051-1.880)	0.202
≥ 60	1		1		1		1	
Marital status								
Single	0.710 (0.042-11.968)	0.812			1.476 (0.087-24.930)	0.787		
Married	1.510 (0.177-12.901)	0.707			1.566 (0.176-13.914)	0.688		
Divorced	1				1			
Education								
Primary school	0.301 (0.029-3.093)	0.313			0.000	0.999		
Secondary school	0.312 (0.030-3.210)	0.328			0.000	0.999		
Bachelor degree	0.000	0.999			1.000 (0.000)	1.000		
Not enrolled	1				1			
Agricultural occupation								
Yes	3.960 (1.148-13.663)	0.029*	7.922 (1.672-37.535)	0.009*	1.060 (0.198-5.672)	0.945		
No	1		1		1			
Smoking								
Daily smoker	0.619 (0.073-5.244)	0.660			1.526 (0.162-14.400)	0.712		
Frequent smoker	0.000 (0.000)	0.999			0.000	1.000		
Occasional smoker	0.000	1.000			0.000	1.000		
Ex-smoker	2.639 (0.627-11.103)	0.186			2.071 (0.216-19.858)	0.528		
Never	1				1			
Alcohol consumption								
Daily drinker	6.136 (1.211-31.088)	0.028*	8.384 (1.061-66.233)	0.044*	27.333 (2.567-291.084)	0.006*	26.990 (1.492-488.156)	0.026*
Frequent drinker	0.000 (0.000)	0.999	0.000 (0.000)	0.998	4.824 (0.287-80.971)	0.274	10.580 (0.389-287.471)	0.161
Occasional drinker	0.662 (0.071-6.133)	0.716	0.860 (0.077-9.589)	0.903	5.857 (0.511-67.098)	0.155	9.197 (0.604-140.148)	0.110
Ex-drinker	3.553 (0.734-17.190)	0.115	4.468 (0.724-27.594)	0.107	0.000 (0.000)	0.999	0.000 (0.000)	0.999
Never	1				1		1	

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Table 3 Univariable and multivariable logistic regression analysis of factors associated with intestinal parasitic infections among participants in 2024 and 2025. (Cont.)

Variables	2024				2025			
	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
Habit of consuming raw or uncooked food								
Yes	1.476 (0.433-5.028)	0.534			7.594 (0.891-64.687)	0.064		
No	1				1			
Walking barefoot on soil								
Yes	1.421 (0.363-5.564)	0.614			1.883 (0.353-10.033)	0.459		
No	1				1			
Handwashing after using toilet								
Yes	0.641 (0.074-5.518)	0.686			0.085 (0.07-1.074)	0.057	0.148 (0.002-9.878)	0.373
No	1				1			
Handwashing after touch pets or stray animals								
Yes	0.117 (0.025-0.541)	0.006*	0.127 (0.018-0.878)	0.036*	0.109 (0.018-0.684)	0.018*	0.420 (0.020-8.610)	0.574
No	1		1		1		1	
Safe drinking water source								
Unsafe	2.115 (0.525-8.520)	0.292			1.373 (0.156-12.094)	0.775		
Safe	1				1			

* *P*-value <0.05 using binary logistic regression, COR = crude odd ratio, AOR = adjust odd ratio.

Data in 2024 were adjust OR with gender, age, agricultural data, alcohol consumption and handwashing after touch pets or stray animals.

Data in 2025 were adjust OR with gender, age, alcohol consumption, handwashing after using toilet and handwashing after touch pets or stray animals.

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4. Discussion

This two-year repeated cross-sectional survey fills an important knowledge gap regarding the epidemiological status of intestinal parasite infections in Thai Samakkhi Subdistrict, Wang Nam Khiao District, a highland rural area northeastern of Thailand where data have been limited. The total prevalence was low (5.19%) and showed a decline from 6.01% in 2024 to 4.27% in 2025, indicating a decreasing trend in transmission during the study period. The low prevalence of intestinal parasite in the present study may be due to the treatment for infection after result notification, enhanced awareness and better preventive behavior in the communities. This result is congruent with previous reported in 2023 as total prevalence 5.78% in Dan Khun Thot, Kham Thale So, Sung Noen and Sikhio Districts of Nakhon Ratchasima and Mueang District of Chaiyaphum Provinces.[14] Our finding is higher than those previously reported for other parts of Thailand, with the 4.24% reported in central region of Thailand in 2006.[18] However, the overall prevalence of intestinal parasite in our study is lower than that reported from northeast Thailand, with 10% overall prevalence in Khon Kaen Province in 2023,[4] and up to 37.2% from rural communities of same province in 2013.[19] Although the prevalence observed in this study was lower than the overall prevalence of 9.79% reported by the Ministry of Public Health of Thailand in 2019,[20] higher rates of possible soil-transmitted helminths (STHs) and *Opisthorchis viverrini* infection suggest that helminths remain the predominant parasitic group in northeastern Thailand.

The predominance of soil-transmitted helminths, particularly hookworms and *Strongyloides stercoralis*, supports previous evidence that STHs remain the most common helminths in northeastern Thailand. The cool, humid and forested environment of Wang Nam Khiao District provides favorable conditions of life cycle of STHs and hatching.[21] Likewise, those parasites are common frequent exposure of soil among agricultural communities such as people living in humid highland of rural northeastern Thailand. Importantly, the molecular confirmation of *Opisthorchis viverrini* in 2025 represents the first district-level evidence of infection in this highland area, where previous surveys had reported no cases.[22] As a result, evidence of *O. viverrini* infection in the area has been mostly based on regional epidemiological surveys rather than district- level studies. *O. viverrini* was molecularly identified in Wang Nam Khiao District with 0.61% in 2025 in this study. This result provides evidence of infection in this highland location. Molecular verification was necessary to distinguish *O. viverrini* from minute intestinal flukes with NADH dehydrogenase subunit 5 (nad5) target gene sequences region,[14, 17] underscoring the importance of incorporating molecular diagnostics in endemic areas. This finding highlights the limitation of morphology-based diagnosis alone and supports the integration of molecular tools into routine surveillance in areas where minute intestinal flukes coexist. Result from the national survey in 2019 also reported that hookworm and *O.viverrini* were two predominant helminthiases with 4.47% and 2.2%, respectively. In addition, Protozoa were also detected, although with a low prevalence (2.02%) with *Blastocystis* spp. being the most frequent species (1.15%) which often involved with coinfections with *Entamoeba coli* or *Pentatrichomonas hominis*, reflecting fecal oral exposure patterns common in agricultural communities.[23]

In this study, the integration of multiple diagnostic methods including direct smear, concentration technique (FECT or EECT), and mAPC enhanced overall sensitivity for parasite detection. To enable molecular analysis in 2025, FECT was replaced with EECT because ethanol-based fixation avoids formalin-induced DNA cross-linking and fragmentation while maintaining diagnostic sensitivity comparable to FECT for eggs, cysts, and larvae.[24, 25] Notably, mAPC demonstrated high sensitivity for detecting *Strongyloides*

stercoralis,[15] which is often under-detected by conventional concentration techniques due to intermittent larval shedding, supporting its inclusion for optimal case detection.

Environmental and lifestyle condition seemed to strongly influence infection patterns.[13] Wang Nam Khiao District has a long cool season, moist climate and vast agricultural areas that sustain the survival of infective parasite stages, thus facilitating continuing transmission even at low prevalence. Agricultural work was a risk factor for infection in 2024 which aligns with the predominance of hookworm, but this association was not presented in 2025. This change may represent the overall decrease in prevalence, less exposure or changes in community hygiene activities during intervals. In addition, participants identified as infected in 2024 were informed of their status and advised to seek appropriate treatment, which may have contributed to the lower infection rates observed in 2025. Daily alcohol intake and handwashing after contact with pets or stray animals were the two behavioral factors significantly associated with infection in both years. Daily alcohol intake likely increases the risk of infection thought several way including sharing raw or undercooked dishes while drinking, reduced hygiene vigilance, and immune suppression associated with chronic alcohol use.[26] This aligns with previous studies reporting that farm workers with soil contact are at higher risk for STH infections. Consistent with our findings, behaviors such as daily alcohol consumption and smoking have been identified as risk factors in rural Thailand, whereas proper handwashing has been shown to reduce the risk of infection.[27, 28] The association between raw food consumption and infection in 2025 may also help explain the detection of *O. viverrini* during that year.[29] Hand hygiene after animal contact remained an important protective factor in both years. Proper handwashing may reduce the risk of indirect hand-to-mouth transmission, particularly in rural settings where free-roaming animals can carry parasite eggs, larvae, and protozoan cysts on their fur and paws,[30] and where environmental conditions favor the survival of infective stages.

Overall, the study highlights a complex interaction between environmental suitability, agricultural activities, and behavioral exposures in determining parasitic infection patterns in a rural region in northeastern Thailand. By establishing baseline information in an area of limited previous knowledge and identifying potentially modifiable behavioral determinants, these findings highlighted the need for ongoing surveillance, molecular diagnosis and community-based health promotion.

This study has several limitations. As cross-sectional surveys, we could not determine the timing of behaviors relative to infection. Self-reported data (e.g., hygiene practices, eating habits) may be subject to recall bias. The sample size limited the precision of estimates for low-prevalence risk factors, highlighting the need for future studies with larger cohorts to confirm these associations. Field-based mAPC processing, which involved transport under cooled conditions (ice packs) may have slightly affected parasite viability, potentially reducing larval recovery and leading to minor underestimation of the true prevalence. In addition, molecular diagnostics were conducted only on a subset of parasites, that may not be representative of the entire community infections. Longer acting studies and expanded molecular analyses are required to better understand transmission dynamics and parasite diversity.

Despite limited evidence for parasite interventions, public health strategies should prioritize sanitation, safe food handling practices and education of high-risk groups such as daily alcohol drinkers should be the main focus of public health strategies. Regular parasitological monitoring, as in Wang Nam Khiao District remains essential for informing effective control strategies.

5. Conclusion

This research reports on the two-year prevalence of intestinal parasitic infections in the study area. Hookworm is the dominant species of helminthic infections in Thai Samakkhi populations in the Wang Nam Khiao District of Nakhon Ratchasima Province. Two behavioral risks were identified including daily drinking and proper hand hygiene after animal contact. These findings suggest that in the rural highlands, infection trends may be diminished and long-term control may be more feasible. Ongoing monitoring and any immediate intervention targeting hygiene education, alcohol reduction programs, safe eating practices or environmental control are required.

6. Acknowledgements

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