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Waterborne parasite contamination and risk factors in natural water sources of Karen communities, Omkoi District, Thailand

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Abstract. *Wongthonglua K, Yanola J, Sanit S, Kongta N, Nachaiwieng W. 2025. Waterborne parasite contamination and risk factors in natural water sources of Karen communities, Omkoi District, Thailand. Biodiversitas 26: 5955-5963.* The Karen hill tribes in Omkoi District, Chiang Mai Province, Thailand, continue to experience a high burden of intestinal parasitic infections, likely linked to the use of untreated mountain water for daily consumption. This study investigated protozoan contamination in natural water sources and identified physicochemical factors associated with contamination risk. Water samples were collected from 30 natural sources during the dry (30 samples) and wet seasons (28 samples), totaling 58 samples. Each 20 L sample was filtered, concentrated, and examined using flotation, microscopy, nested PCR to detect *Entamoeba histolytica* and *Entamoeba coli*, and the addition of DNA sequencing to detect *Giardia duodenalis* and *Cryptosporidium* spp.. Physicochemical parameters, including pH, turbidity, total dissolved solids (TDS), dissolved oxygen, ammonia, chlorine, nitrate, and nitrite, were also measured. Fisher's exact tests and logistic regression were applied to evaluate associations with contamination. To our knowledge, this study represents the first molecular assessment of protozoan parasites in Omkoi community water sources. *E. histolytica* was detected in two sources per season (6.67% dry; 7.14% wet), and *E. coli* in one source per season (3.33% dry; 3.57% wet). *G. duodenalis* and *Cryptosporidium* spp. were not detected. Significant associations were observed between contamination and elevated TDS (≥200 mg/L; OR = 29.33; 95% CI = 2.08-423.66), ammonia (≥0.5 mg/L; OR = 25.50; 95% CI = 1.88-345.83), and nitrite (>0.1 mg/L; OR = 51.00; 95% CI = 4.00-649.10) (P<0.05). Although these parameters are not visually detectable, green algal blooms may indicate nutrient-rich conditions conducive to contamination. In conclusion, routine water treatment, such as boiling or filtration, is recommended before consumption. Residents should avoid water sources with visible algal growth to reduce the risk of protozoan infection in these rural communities.

Keywords: Karen hill tribe, waterborne parasites, natural water sources, water quality, parasitic contamination

INTRODUCTION

Waterborne parasites such as *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* remain persistent global public health concerns. These protozoan parasites are transmitted primarily through contaminated water sources and represent major challenge for communities lacking adequate water treatment and sanitation facilities. *G. duodenalis* is one of the most common causes of protozoal diarrhea worldwide, producing giardiasis that manifests in abdominal cramps, nausea, and chronic diarrhea (Cernikova et al. 2018). Its cyst form is resistant to conventional chlorination, making eradication through standard water treatment difficult (Martins Moutinho et al. 2024). Similarly, *Cryptosporidium* spp. produces highly resistant oocysts that persist in diverse environmental conditions and are associated with cryptosporidiosis, disease that can cause severe diarrhea, fever, and dehydration, particularly in children and immunocompromised individuals (Martins Moutinho et al. 2024). Another important protozoan, *E. histolytica*, is transmitted through ingestion of infective cysts in contaminated water or food. Infection can lead to amoebiasis, which ranges from mild gastrointestinal disturbances to severe conditions such as amoebic liver abscesses (Tharmaratnam et al. 2020). Together, these parasites underline the importance of safe water supply and adequate sanitation infrastructure in preventing outbreaks. Prior research has emphasized that water quality improvement, sanitation development, and hygiene promotion (WASH interventions) provide sustained and effective mechanisms for reducing the incidence of diarrheal diseases (Chaysiri et al. 2024).

In low-income and geographically isolated communities, these challenges are often intensified due to limited access to public utilities. Omkoi District in Chiang Mai Province, northern Thailand, provides a notable example. Recognized as one of the most impoverished areas in the region, Omkoi is largely inhabited by the Karen hill tribe, which accounts for more than 90% of the population. The district is situated in a rugged mountainous area, where steep slopes and difficult terrain impede infrastructure development and limit access to essential services such as piped water supply and wastewater management. Because of these constraints, most households rely on natural mountain streams and springs, locally referred to as "mountain water supply," for daily consumption and household use (Sudsandee et al. 2020). These water sources, however, are rarely treated and remain vulnerable to contamination from human and animal activities, soil erosion, and surface runoff, all of which create favorable conditions for the persistence of waterborne parasites (Damitie et al. 2020).

Epidemiological investigations in Omkoi have demonstrated that parasitic infections constitute a serious health problem in the area. A previous study by Yanola et al. (2018) revealed that parasitic infection prevalence could reach as high as 35% among residents, with stool samples confirming the presence of waterborne parasites. These findings highlight the link between an untreated mountain water supply and elevated infection risks. When combined with the socioeconomic conditions of the Karen hill tribe, including poverty, limited healthcare access, and reliance on traditional practices, the situation underscores a cycle of vulnerability where poor water quality contributes to recurrent illness, reduced productivity, and deepened poverty. Importantly, these findings align with global observations that marginalized rural communities are often disproportionately affected by protozoan infections due to their dependence on unsafe drinking water sources (Damitie et al. 2020).

This study was conducted to investigate the extent of waterborne parasite contamination in Omkoi District, focusing on the detection of *G. duodenalis*, *Entamoeba coli*, *E. histolytica,* and *Cryptosporidium* spp. in water sources used by the Karen hill tribe for drinking and domestic purposes. It is also the first research to examine the physical and chemical characteristics of these sources to identify links between water quality indicators and waterborne protozoa occurrence. The findings aim to raise community awareness about the risks of untreated water consumption, support preventive measures to reduce infections, and provide evidence for policymakers to develop targeted, low-cost water treatment and monitoring strategies. By integrating parasitological data with water quality assessments, this research enhances understanding of waterborne parasite transmission in resource-limited mountainous areas and informs sustainable, community-centered health interventions.

MATERIALS AND METHODS

Study site and water sampling procedure

The study site is located in Omkoi District, Chiang Mai Province, Thailand (Figure 1), approximately 187 km southwest of Chiang Mai City, at coordinates 17.6338° N and 98.3527° E, with an elevation of 1,012 m above sea level. A total of fifty-eight water samples were collected from various natural water resources in the Omkoi District. These resources included rivers, ponds, and reservoirs. Thirty samples were taken during the dry season in April 2023 (no rain recorded at least one week preceding the days of collection), and another twenty-eight were taken during the wet season in August 2023 at the same sites (Two water sampling sites could not be reached because of poor road conditions during the wet season). The samples were collected from six sub-districts of Omkoi: Omkoi, Nakian, Yangpiang, Maelong, Monjong, and Maeteun Sub-districts. The number of water samples collected in each area varies with the proportion of households in the area. The collection sites were chosen based on the water resources available for community consumption. The 20 L of surface water sample was collected using the grab sampling technique from a depth of 5 cm and transferred to 20 L plastic containers before being sent to the laboratory.

Ethical approval

This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand (Project Code: AMSEC-68EM-008).

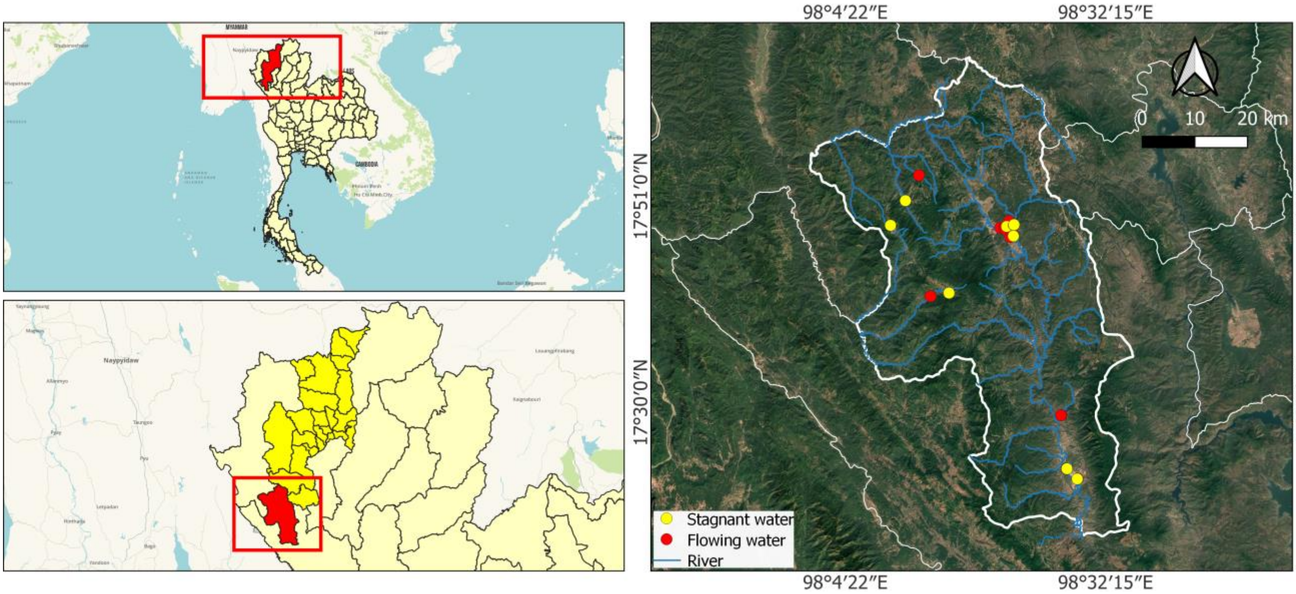

Figure 1. The study site in Omkoi District, Chiang Mai Province, Thailand

Physicochemical parameter measurements

Water flow was first recorded as stagnant or flowing water source at the sampling sites. Physical characteristics were measured for five parameters including pH (ProfiLine pH 3310, WTW, USA), turbidity (Portable turbidity meter Turb® 430 IR, WTW, USA), salinity (SALTTEST11 Salinity Meter, Thermo Scientific, USA), total dissolved solids (TDS) (TDSTestr11 Dual Range TDS Tester, Thermo Scientific, USA), and dissolved oxygen (DO) (HQ40D, Hach, UK). Chemical parameters, including ammonia, chlorine, and nitrite, were measured using the water test kits (V UNIQUE, Thailand), and nitrate was measured by the HI 3874 test kit (Hanna Instruments, USA). We calibrated pH, Electrical Conductivity (EC), DO, and turbidity meters before each sampling day following manufacturer procedures.

Water concentration

The water samples were taken to a laboratory and concentrated by passing them through a cellulose acetate membrane (Sartorius, Goettingen, Germany) with a diameter of 47 mm and a pore size of 1.2 μm using the filtration apparatus (Wheaton, USA). Before filtering the next sample, the filtration apparatus was thoroughly washed with sterile distilled water to ensure that no carryover occurred between samples. The sediment collected on the membrane surface was then flushed out using 100 mL of a 0.1% Tween 80 solution in 10 mM phosphate-buffered saline (PBS). The sediment was then washed twice with 100 mL of 10 mM PBS using centrifugation at 1000 x g. The washed sediment was collected in 50 mL polypropylene centrifuge tubes (Corning, MA) and subjected to the sucrose floating procedure.

Sucrose floatation

A total of 35 mL of sucrose solution (specific gravity 1.2) was added to the washed sediment tube and mixed thoroughly following the prior procedure (Kuczynska and Shelton 1999). The mixture was allowed to stand for 10 min, then centrifuged at 1,000 × g for 10 min. From the upper fraction, 10 mL was collected and diluted with 20 mL of distilled water to adjust the specific gravity to 1.0, followed by centrifugation at 2,700 × g for 15 min at room temperature. The resulting sediment was preserved with 10 mL of 70% ethanol for subsequent DNA amplification (samples from this step were labeled as /F). The remaining 20 mL of solution from the flotation process was retained for further analysis by adding 40 mL of distilled water and repeating the upper fraction flotation process, ending with the addition of 10 mL of 70% ethanol (samples labeled as /S). The sediment from the flotation process was preserved with 10 mL of 70% ethanol (samples labeled as /P).

Microscopic examination of parasites

Each sample collected from the upper fraction (F), middle (S), and sediment fraction (P) of the sucrose flotation procedure was prepared using three techniques: simple smear, trichrome staining, and modified acid-fast staining to detect parasites. The simple smear method was performed by spreading two drops (50 μL) of the flotation specimen onto a clean glass slide, followed by immediate placement of a coverslip to enclose the sample.

Trichrome staining, 50 μL of the specimen was dropped onto a coverslip (22 × 22 mm) and air-dried. The dried smear was fixed in Schaudinn's fixative for 30 min, then dipped into two changes of iodine-alcohol solution for 2 min each, followed by two changes of 70% ethanol for 2 min each. The coverslips were stained with trichrome solution for 12 min. Destaining was carried out using an acetic acid-alcohol solution for 1-2 sec, followed by two rinses with 90% ethanol for 2 sec each. The coverslips were then dehydrated in two changes of 95% and 100% ethanol for 5 min each. After drying on paper towels, the coverslips were cleared in two changes of absolute xylene for 2 min each. Finally, the coverslips were mounted onto glass slides using mounting medium (Permount®, Fisher Chemical, Belgium).

Modified Ziehl-Neelsen acid-fast staining, the dried, methanol-fixed sample was flooded with cold carbol-fuchsin stain for 15 min. Excess stain was rinsed off with tap water, and the slide was decolorized using 1% acid alcohol (1% HCl in 95% ethanol). Malachite green (0.4%) was used as a counterstain and applied for 30 sec. The slides were then mounted using the same mounting medium.

A total of 522 slides were examined throughout the slide at 400× magnification and photographed under a light microscope (Olympus CX41 with DP27 digital camera, Japan) by experienced parasitologists for parasite identification based on morphological characteristics.

Pretreatment and genomic DNA extraction

The pretreatment was carried out following the described method (Babaei et al. 2011) with some modifications. In brief, 200 μL of sediment samples were combined with an equal volume of glass beads (0.45-0.52 mm diameter), followed by mixing for 10 min. The mixture was subjected to 7 freeze/thaw cycles, alternating between liquid nitrogen for 5 min and a 95°C heating box for 7 min. Added 400 μL of InhibitEX Buffer (Qiagen, Hilden, Germany), followed by vortexing continuously for 15 min and incubating at 70°C for 5 min. The suspension was centrifuged at 13,000 x g for 1 min, and 200 μL of the supernatant was transferred to a 2 mL sterile tube. After adding 15 μL of proteinase K and 200 μL of DNA lysis buffer (Qiagen, Hilden, Germany), the sample was gently vortexed and incubated at 70°C for 10 min. The lysate was subjected to genomic DNA extraction using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. All genomic DNA was stored at -20°C until use. Positive controls (cysts/oocysts) of the four target parasite strains were isolated from clinical stool specimens and kindly provided by the Department of Parasitology, Faculty of Medicine, Chiang Mai University. The preparation of positive controls followed the same protocol as that used for sample processing. A field blank and an extraction blank were processed per batch, and a no-template control (NTC) was included in every PCR run.

Nested PCR amplification and DNA sequencing of the fragment of *β-giardin* gene of *Giardia duodenalis*

The fragment of *β-giardin* gene of *G. duodenalis* was amplified using outer primer pair, GD-1 F and GD-1 R, and inner primer pair, GD-2 F and GD-2 R (Table 1) (Cacciò et al. 2002; Lalle et al. 2005). PCR reactions were carried out in a volume of 20 µL with a final concentration of 4 U of Platinum taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 0.5 µM each of the forward and reverse primers. The first round PCR amplification consisted of an initial heat activation step at 94°C for 2 min, followed by 35 cycles of 94ºC for 45 sec, 65°C for 45 sec, and 72°C for 60 sec with a final extension step at 72ºC for 5 min. The second round PCR amplification consisted of an initial heat activation step at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 60 sec with a final extension step at 72ºC for 5 min. PCR products were analyzed by electrophoresis on 1.7% agarose gel (Invitrogen, Carlsbad, CA, USA) using a voltage of 120 V for 25 min and visualized under UV light by RedSafe™ Nucleic Acid Staining (iNtRON biotechnology, Korea) using GelDocXR+ (Biorad). PCR product was then purified and sequenced in both directions (Macrogen, South Korea). Nucleotide sequences were aligned using Geneious Prime 2023.1.2 (https://www. geneious.com).

Nested PCR amplification and DNA sequencing of the fragment of the 18S rDNA gene of *Cryptosporidium* spp.

The fragment of the *18S rDNA* gene of *Cryptosporidium* spp. was amplified using outer and inner primers according to the previous report (Ryan et al. 2003) (Table 1). PCR reactions were carried out in a volume of 20 µL with a final concentration of 4 U of Platinum *taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 0.5 µM each of the forward and reverse primers. The first and second rounds of PCR amplifications consisted of an initial heat activation step at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 58°C for 45 sec, and 72°C for 60 sec with a final extension step at 72°C for 5 min. PCR products were analyzed by electrophoresis on a 1.7% agarose gel (Invitrogen, Carlsbad, CA, USA) using a voltage of 120 V for 25 min and visualized under UV light by RedSafe™ Nucleic Acid Staining (iNtRON biotechnology, Korea) using GelDocXR+ (Biorad). The PCR product was then purified and sequenced in both directions (Macrogen, South Korea). Nucleotide sequences were aligned using Geneious Prime 2023.1.2 (https://www. geneious.com).

Detection of *Entamoeba histolytica* and *Entamoeba coli* using nested PCR amplification

A nested PCR amplification targeting the *E. histolytica*-18S rRNA gene was performed to detect *E. histolytica.* Two sets of primers, *Entamoeba* genus-specific primers and *E. histolytica* species-specific primers, were used as previously described (Khairnar and Parija 2007). The first round PCR for genus-specific amplification reactions was carried out in a volume of 20 µL with a final concentration of 4 U of Platinum taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 0.5 µM each of the forward and reverse primers. The first round PCR for genus-specific amplification consisted of an initial heat activation step at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 56°C for 45 sec, and 72°C for 60 sec with a final extension step at 72°C for 5 min. The second round PCR for species-specific amplification consisted of an initial heat activation step at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 48°C for 45 sec, and 72°C for 60 sec with a final extension step at 72°C for 5 min.

Table 1. Primer sequences for amplifications of *Giardia duodenalis*-β-giardin and *Cryptosporidium* spp. -18S rDNA genes and detections of *Entamoeba histolytica* and *Entamoeba coli* targeting 18S rRNA genes

Protozoa	PCR amplification	Forward primer sequence	Reverse primer sequence	Product size (bp)
Nested PCR amplifications of *Giardia duodenalis*-β-giardin and *Cryptosporidium* spp.-18S rDNA genes				
Cryptosporidium spp.	1st round PCR	18SiCF2: 5′-GAC ATA TCA TTC AAG TTT CTG ACC-3′	18SiCR2: 5′-CTG AAG GAG TAA GGA ACA ACC-3′	763
	2nd round PCR	18SiCF1: 5′-CCT ATC AGC TTT AGA CGG TAG G-3′	18SiCR1: 5′-TCT AAG AAT TTC ACC TCT GAC TG-3′	587
Giardia duodenalis	1st round PCR	GD1-F: 5′-AAG CCC GAC GAC CTC ACC CGC AGT GC-3′	GD1-R: 5′-GAG GCC GCC CTG GAT CTT CGA GAC GAC-3′	753
	2nd round PCR	GD2-F: 5′-GAA CGA GAT CGA GGT CCG-3′	GD2-R: 5′-CTC GAC GAG CTT CGT GTT-3′	511
Detections of *Entamoeba histolytica* and *Entamoeba coli* targeting 18S rRNA genes				
Entamoeba coli	1st round PCR[a]	Entam1: 5′-GTT GAT CCT GCC AGT ATT ATA TG-3′	Entam2: 5′-CAC TAT TGG AGC TGG AAT TAC-3′	550
	2nd round PCR[b]	EcoliF: 5’- CTA AGC ACA AAG TCC TAG TAT GAT G -3’	EcoliR: 5’-CCT CAT CGA TTA CAC TCC CAG AG-3’	166
Entamoeba histolytica	1st round PCR[a]	E-1: 5'-TAA GAT GCA CGA GAG CGA AA-3'	E-2: 5'-GTA CAA AGG GCA GGG ACG TA-3'	898
	2nd round PCR[b]	EH-1: 5'-AAG CAT TGT TTC TAG ATC TGA G-3'	EH-2: 5'-AAG AGG TCT AAC CGA AAT TAG-3'	439

Note: [a] The 1st round PCR for genus-specific amplification and [b] The 2nd round PCR for species-specific amplification

To detect *E. coli,* nested PCR amplification targeting *E. coli*-18S rRNA gene was carried out using two sets of primers, *Entamoeba* genus-specific primers (Verweij et al. 2001) and *E. coli* species-specific primers (Rattaprasert et al. 2022), as previously described (Table 1). PCR reactions were performed in a volume of 20 µL with a final concentration of 4 U of Platinum taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 0.5 µM each of the forward and reverse primers. The first and second rounds of PCR amplifications consisted of an initial heat activation step at 94°C for 2 min, followed by 35 cycles of 94°C for 45 sec, 55°C for 45 sec, and 72°C for 60 sec with a final extension step at 72°C for 5 min. PCR products were analyzed by electrophoresis on a 1.7% agarose gel (Invitrogen, Carlsbad, CA, USA) using a voltage of 120 V for 25 min and visualized under UV light by RedSafe™ Nucleic Acid Staining (iNtRON biotechnology, Korea) using GelDocXR+ (Biorad).

Statistical analysis

The physicochemical characteristics of water resources in the Omkoi area were summarized using mean values and standard deviations. Independent T-tests were performed to compare the means across sampling seasons and water flow conditions. Fisher's exact test was applied to examine the association between physicochemical characteristics and waterborne parasite contamination. Statistical significance was set at $P<0.05$. Logistic regression analysis was conducted to identify risk factors associated with waterborne parasite contamination. Because positive samples were rare, a parsimonious logistic model adjusted for season and flow was used, and odds ratios with 95% confidence intervals and events per category were reported. Fisher's exact tests were also provided in the supplementary material as sensitivity analyses. All statistical analyses were performed using SPSS version 29.0.1.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Water physicochemical analysis

Marked seasonal differences in general characteristics of water sources were observed (Figure 2). During the wet season, water levels were higher, and turbidity increased compared to the dry season. Furthermore, the physicochemical characteristics of water resources in the Omkoi area also varied throughout the sampling season. All parameters, except for turbidity, showed significant differences ($P<0.05$), including dissolved oxygen (DO), total dissolved solids (TDS), pH, salinity, ammonia, nitrate, and nitrite. Chlorine was not detected in Omkoi water resources. During the same sampling season, the physicochemical characteristics of both stagnant water and flowing water resources were similar in the dry season and slightly different in pH ($P = 0.011$) and ammonia concentration ($P = 0.042$) in the wet season (Table 2).

Figure 2. Comparison of water source characteristics at the same location in Omkoi. A. Wet season, B. Dry season

Table 2. The physicochemical characteristics of water samples collected from natural water resources in Omkoi District

Sampling Season	Water flow	Number of sample	Average and SD	Physicochemical of water								
				Physical					Chemical			
				DO (mg/L)	TDS (mg/L)	Turbidity (NTU)	pH	Salinity (ppt)	Ammonia (mg/L)	Chlorine (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)
Dry	Stagnant water	8	Average	7.93	98.75	93.29	6.04	0.05	0.25	0.00	10.00	0.04
			SD	3.13	99.35	183.33	0.80	0.07	0.14	0.00	0.00	0.06
	Flowing water	22	Average	6. 33	100.91	162.85	5.90	0.08	0.28	0.00	10.45	0.42
			SD	0.92	60.47	204.80	0.52	0.05	0.38	0.00	2.13	0.10
T (water flow)				1.428	-0.72	-0.844	0.567	-1.339	-0.227	-	-0.596	0.018
P (water flow)				0.194	0.943	0.406	0.575	0.191	0.822	-	0.556	0.986
Wet	Stagnant water	5	Average	2.40	63.80	41.14	6.90	0.00	0.24	0.00	1.80	0.03
			SD	0.74	84.24	32.17	0.18	0.00	0.22	0.00	1.64	0.01
	Flowing water	23	Average	3.07	42.65	242.87	7.27	0.00	0.09	0.00	1.48	0.04
			SD	0.97	38.03	296.43	0.31	0.00	0.14	0.00	2.15	0.05
T (water flow)				-1.438	0.549	-3.179	-2.557	-	1.967	-	0.313	-0.339
P (water flow)				0.162	0.610	0.004	0.017	-	0.060	-	0.756	0.687
T (Season sampling variation)				9.666	3.367	-0.977	-10.144	6.886	2.231	-	17.236	0.414
P (Season sampling variation)				<0.001	0.001	0.333	<0.001	<0.001	0.030	-	<0.001	0.681

Note: T stands for the T-test and P stands for the P-value

Microscopic examination

None of the obvious parasites were observed in the 522 slides of 58 samples using simple smear, trichrome stain, and modified acid-fast stain; however, the cyst-like suspected organisms resembling to *Entamoeba* spp. with a similar size, approximately 10 µm, from locations 2, 3, and 5 were detected (Figure 3). Therefore, further identification by molecular analysis was performed.

Molecular detection of waterborne parasites

A total of 116 water samples were prepared from 30 natural water sources during the dry (60 samples; 30 samples each from /S+/F and /P fraction) and wet (56 samples; 28 samples each from /S+/F and /P fraction) seasons to detect four waterborne parasites, *G. duodenalis*, *Cryptosporidium* spp., *E. histolytica*, and *E. coli* by using molecular analysis. Only *E. histolytica* and *E. coli* were detected (Figure 4), found in six samples (5.17%) from four locations (locations 2, 3, 13, and 15) across both seasons. During the dry season, *E. histolytica* was detected in 3.33% of samples, and *E. coli* in 1.67%. In the wet season, *E. histolytica* and *E. coli* were identified in 3.57% and 1.78% of samples, respectively. All detections in both seasons originated from three sampling locations (Table 3).

Among 30 natural water sources sampled from Karen hill tribe areas in Omkoi District, Chiang Mai Province, *E. histolytica* was detected in two sources per season (6.67% in dry and 7.14% in wet season), and *E. coli* in one source per season (3.33% in dry and 3.57% in wet season) (Table 3).

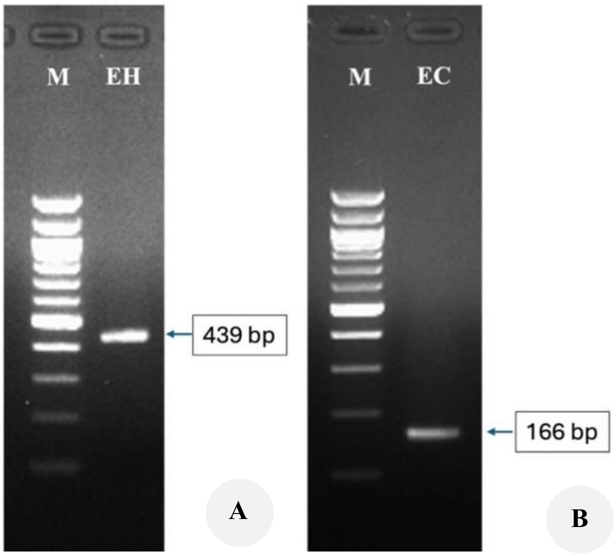

Figure 4. Agarose gel electrophoresis results of nested PCR amplification for detection of waterborne parasite DNA from genus-specific primers. A. *Entamoeba histolytica* (EH), product size 439 bp, B. *Entamoeba coli* (EC), product size 166 bp; M: 100 bp marker

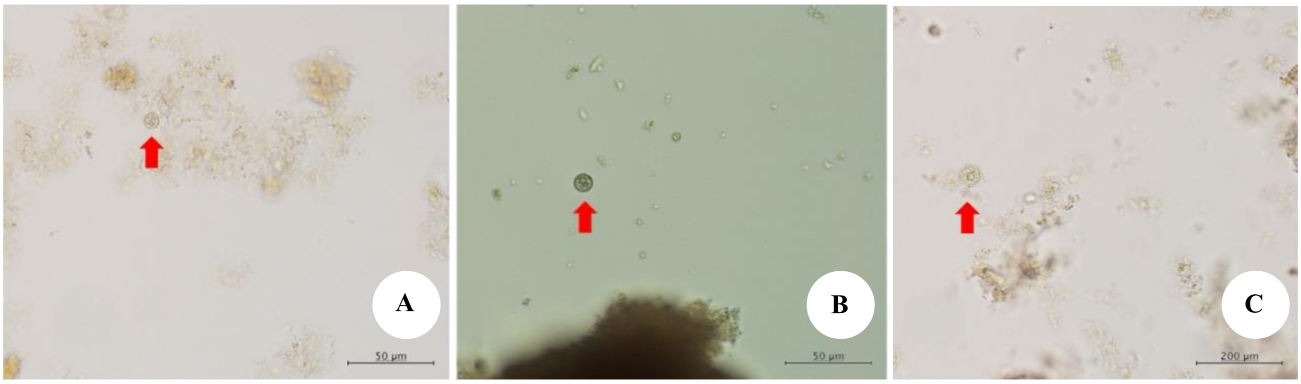

Figure 3. The suspected microorganisms resembling *Entamoeba*-like cysts from location A. 2, B. 3, C. 5, under the simple smear microscopic examination

Table 3. Detection of four waterborne parasites in 30 collected locations of Karen hill tribe natural water sources in dry and wet seasons from Omkoi District, Chiang Mai Province

Waterborne parasites	Number of positive sample using molecular analysis		Total number of location which found positive samples
	Dry season (60 tested samples)	Wet season (58 tested samples)	
Giardia duodenalis	0	0	0
Cryptosporidium spp.	0	0	0
Entamoeba histolytica	2 (sample: 2D/S+F, 15D/S+F)	2 (sample: 13W/P, 15W/S+F)	3 (location: 2, 13, 15)
Entamoeba coli	1 (sample: 3D/S+F)	1 (sample: 2W/S+F)	2 (location: 2, 3)

Note: Sample IDs are formatted as (location)(season)/(fraction from sucrose flotation procedure)

The correlation between water physicochemical characteristics and the contamination of parasites in the water resources

Physicochemical data of water sources were categorized into two groups, contaminated and non-contaminated, in order to examine the relationship between water quality parameters and the presence of parasitic contamination. It was discovered that the TDS (P = 0.019), ammonia (P = 0.026), and nitrite (P = 0.002) were significantly correlated with waterborne parasite contamination at P<0.05. Furthermore, logistic regression indicated that the TDS (P = 0.013), ammonia (P = 0.015), and nitrite (P = 0.002) were identified as risk factors and had a significant association with waterborne parasite contamination in the water. The risk of waterborne parasite contamination is 29.33 times higher in high TDS water (≥200 mg/L) compared to low TDS water (<100 mg/L). Water with high ammonia concentration (≥0.5 mg/L) shows a 25.50 times higher risk of waterborne parasite contamination than water with lower ammonia concentration. Additionally, water containing high nitrite levels (≥0.1 mg/L) shows a 51.00 times higher risk of contamination (Table 4).

Discussion

This research is very important because there is a high prevalence of parasitic infections among the Karen hill tribe population in the Omkoi area. A previous study found that the prevalence of intestinal parasitic infections among the Karen population in Omkoi District was over 35%, some of them were found to be waterborne parasites and are probably not decreasing very soon (Yanola et al. 2018). The *E. coli*, a fecal indicator organism, and *E. histolytica* detected in this study are consistent with the strains commonly found to infect local villagers (Yanola et al. 2018; Nachaiwieng et al. 2024). Moreover, according to observations in the Omkoi area, most of the population, who lack a piped water system, use untreated water from natural sources for their drinks and other consumption. This is very impactful when the water is contaminated with waterborne parasites.

Table 4. The relationship and risk factors between water physicochemical characteristics and the contamination of parasites in the water resources

Water characteristics	Water sample n (%)	Waterborne parasites		Fisher's exact	P-value[a]	OR	P-value[b]	95% CI
		Not found n (%)	Found n (%)					
Water flow								
Stagnant water	13 (22.40)	12 (92.30)	1 (7.70)	0.00	1.000	0.67	0.723	0.71-6.27
Flowing water	45 (77.60)	42 (89.40)	5 (10.60)			1.00		
Sampling season								
Dry	30 (51.70)	27 (90.00)	3 (10.00)	0.00	1.000	0.93	0.929	0.17-5.01
Wet	28 (48.30)	25 (89.30)	3 (10.70)			1.00		
pH								
<7	36 (62.10)	31 (86.10)	5 (13.90)	0.00	0.392	3.39	0.281	0.37-31.10
≥7	22 (37.90)	23 (95.80)	1 (4.20)			1.00		
Turbidity (NTU)								
Low (<25)	21 (36.20)	19 (82.60)	4 (17.40)	2.41	0.313	5.18	0.158	0.53-50.65
Moderate (25-100)	14 (24.10)	13 (92.90)	1 (7.10)			1.69	0.718	0.10-29.41
High (≥100)	23 (39.70)	22 (95.70)	1 (4.30)			1.00		
TDS (mg/L)								
1-99	47 (81.00)	45 (93.80)	3 (6.30)	7.40	0.019*	1.00		
100-199	8 (13.80)	8 (88.90)	1 (11.10)			2.10	0.546	0.19-23.09
≥200	3 (5.20)	1 (33.30)	2 (66.70)			29.33	0.013*	2.08-423.66
DO (mg/L)								
<4	22 (37.90)	20 (87.00)	3 (13.00)	0.00	0.664	1.74	0.524	0.32-9.48
≥4	36 (62.10)	34 (91.90)	3 (8.10)			1.00		
Salinity (ppt)								
<0.1	38 (65.50)	36 (90.00)	4 (10.00)	0.00	1.000	1.06	0.950	0.18-6.35
≥0.1	20 (34.50)	18 (90.00)	2 (10.00)			1.00		
Ammonia (mg/L)								
0.0-0.5	55 (94.80)	53 (93.00)	4 (7.00)	0.00	0.026*	1.00		
≥0.5	3 (5.20)	1 (33.30)	2 (66.70)			25.50	0.015*	1.88-345.83
Nitrate (mg/L)								
0-9.0	28 (48.30)	27 (90.00)	3 (10.00)	0.00	1.000	1.00		
≥9.0	30 (51.70)	27 (90.00)	3 (10.00)			0.929	0.926	0.17-5.02
Nitrite (mg/L)								
0-0.1	54 (93.10)	53 (94.60)	3 (5.40)	20.12	0.002*	1.00		
≥0.1	4 (6.90)	1 (25.00)	3 (75.00)			51.00	0.002*	4.00-649.10

Note: *significant at P<0.05; [a] refers to the P-value of Fisher's exact test; [b] refers to the P-value of logistic regression. The cut-offs for each parameter follow the manufacturer's guidelines

Limited funding restricted our sampling to 30 sites in Omkoi District. Although we attempted to detect waterborne parasites with a simple microscopic technique, we were unable to clearly identify their characteristics. We tried three different slide methods with the concentrated sample, but the sensitivity of the microscopic method might have been low due to the microscopist's skill and experience in identifying the environmental parasites (Zarlenga and Trout 2004). As a result, the more sensitive technique was recommended (El-Badry et al. 2010). Several studies have reported a high rate of waterborne parasite contamination in the water resources of Thailand, including *Cryptosporidium* spp. (12.7-26.9%) and *G. duodenalis* (7.6-21.5%) (Srisuphanunt et al. 2010; Kumar et al. 2016). These were detected using immunomagnetic separation (IMS) and fluorescent microscopic examination, which is a standard method recommended by the United States Environmental Protection Agency (USEPA) (Method 1623.1). However, due to budget constraints, this study used nested PCR, an alternative molecular technique for detecting waterborne parasites. Although nested PCR has lower sensitivity compared to IMS, it may lead to missed detection of low concentrations of *G. duodenalis* and *Cryptosporidium* spp., it is still an accepted method for detecting waterborne parasites by various studies (Mayer and Palmer 1996; Chuah et al. 2016).

According to the findings of this study, *E. coli* was detected at sampling sites 2 (wet season) and 3 (dry season), whereas *E. histolytica* was detected at site 15 during both the wet and dry seasons, as well as at site 2 (dry season) and site 13 (wet season). To our knowledge, this is the first molecular detection of *Entamoeba* spp. in community water sources in Omkoi. Consistent with the findings of Chuah et al. (2016), our results show a higher frequency of waterborne parasite detection during the wet season, underscoring the role of water as a transport agent. The parasite-contaminated locations are situated downstream and are connected to water sources that traverse residential areas. Domestic wastewater discharges likely contaminate these sites. These observations are consistent with previous studies indicating the presence of *E. histolytica* in water sources contaminated by human and animal excreta (Pham Duc et al. 2011). Furthermore, analysis of the water quality at the positive sampling sites during both the wet and dry seasons revealed that some locations exhibited low DO levels (<4 mg/L) and elevated ammonia concentrations (≥0.5 mg/L). These values fall below the acceptable class III (fairly clean freshwater used for consumption and for industries) national surface water quality standard and may indicate contamination by protein-rich waste (Yadav et al. 2019).

The analysis of risk factors linking physicochemical characteristics to waterborne parasite contamination is valuable and aligns with previous studies predicting risks associated with water consumption (Onichandran et al. 2013, 2014; Ligda et al. 2020). In this study, ammonia and nitrite were the key chemical components associated with parasite detection, reflecting human and animal waste decomposition—consistent with earlier findings showing strong correlations between protozoa and organic pollutants (Onichandran et al. 2014). Because villagers lack the tools and knowledge to measure these parameters, observable indicators of contamination are essential. High TDS (≥200 mg/L), ammonia (≥0.5 mg/L), and nitrite (≥0.1 mg/L) indicate high consumption risk, although wide 95% CIs in Table 4 suggest low precision due to small sample sizes, requiring cautious interpretation and further validation. Previous studies show that flowing water tends to have lower contamination, whereas stagnant or slow-moving water bodies accumulate pathogens (Sarma 2020). Although chemical pollutants cannot be directly seen, algal blooms can signal elevated ammonia and nitrite levels. The recommendation to use visible indicators for community awareness is especially useful in low-resource settings. Nevertheless, natural water must be treated before consumption through filtration or methods such as chlorination, UV, or ozonation (Sarma 2020). This research model can also be applied to other regions relying on untreated water, and the findings have been shared with local health authorities to support planning and improvement of water systems in resource-limited communities.

In conclusion, waterborne parasites, including *E. histolytica* (6.67% in dry and 7.14% in wet season), and *E. coli* in one source per season (3.33% in dry and 3.57% in wet season), have been detected by the nested PCR technique in natural water resources in the Omkoi area. Monthly deployment of field blanks and validation of IMS/IF microscopy over a 12-month time series at all positive sites should be performed to ensure reliability of the reported data. The risk factors for waterborne parasite contamination are TDS, ammonia, and nitrite concentration. Water with high TDS (≥200 mg/L) has a 29.33 times higher risk of waterborne parasite contamination compared to water with lower TDS. Furthermore, water containing high levels of ammonia (≥0.5 mg/L), and nitrite (≥0.1 mg/L) poses a 25.50- and 51.00-times higher risk of contamination, respectively. To reduce exposure, it is recommended to avoid consuming water with visible green algal blooms or to apply pretreatment methods such as boiling, filtration, and chemical disinfection. Our findings were shared with the local public health authority to support the development of appropriate health policy strategies, such as routine parasitic monitoring among villagers and initiatives to improve water quality within Karen communities.

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