

Prevalence and Distribution of Protozoan Parasite Contamination in Fresh Vegetables from Bang Khla District, Chachoengsao Province, Thailand

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Abstract

Intestinal protozoan infections pose a significant public health concern, particularly for children and the elderly. This cross-sectional study investigated the prevalence and distribution of protozoan cysts and oocysts in fresh vegetables grown in Bang Khla District, Thailand. The analysis employed the sedimentation technique and modified acid-fast staining method to analyze a total of 369 samples encompassing nine commonly consumed vegetable types collected from cultivation plots. The prevalence of four protozoan species was detected: *Cryptosporidium* spp. (11.9%), *Entamoeba histolytica*/*E. dispar* (7.6%), *Blastocystis* sp. (3.5%), and *Giardia* spp. (1.9%). Notably, there was a statistically significant variation in contamination rates across different types of fresh vegetables ($p < 0.05$). Among the nine vegetable types, water spinach exhibited the highest contamination rate (70.7%), followed by coriander (36.6%), lettuce (34.1%), celery and kale (each at 22.0%), hoary basil and sweet basil (each at 12.2%), spring onion (9.8%), and yardlong bean (4.9%). The high contamination observed in water spinach, a semi-aquatic plant, suggests a potential role of irrigation water as an important source of protozoan contamination. The consumption of raw vegetables, particularly water spinach, may therefore pose a significant risk for the transmission of protozoan infections. These findings underscore the importance of food safety practices, including proper washing and cooking, as well as improved management of irrigation water and cultivation environments. This study provides important evidence supporting the need for strengthened environmental sanitation, targeted public health interventions, and further research on contamination pathways in agricultural systems.

Keywords: protozoan contamination, fresh vegetables, food safety, irrigation contamination, water spinach

Introduction

Intestinal protozoan infections remain a significant public health concern, particularly with a noticeable impact on the health of children and the elderly. These protozoan parasites, with a global reach that stretches across tropical and subtropical regions, place a substantial burden on healthcare systems and individual health (Berhe et al., 2018). Worldwide, approximately 3.5 billion people are infected with intestinal protozoan parasites. Epidemiologically, parasitic protozoan outbreaks contribute to 1.7 billion annual episodes of diarrhea, with intestinal protozoa serving as the source of numerous foodborne diseases (Eslahi et al., 2023; Li et al., 2020). Protozoan parasites have the potential to cause severe diseases in the human intestinal tract. The three most commonly found intestinal protozoan pathogens that can infect humans and cause serious diseases are *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium parvum* (Hemphill et al., 2019; Suleiman & Azlan, 2025). *C. parvum* and *G. lamblia* inhabit the duodenum, jejunum, and ileum of the human intestinal tract. They are the main causes of ongoing diarrhea, especially conditions known as cryptosporidiosis and giardiasis. In contrast, *E. histolytica* colonizes the colon and can invade the colon wall and spread to other organs, mainly the liver, causing life-threatening amebiasis (Hemphill et al., 2019). Protozoan infections can cause various symptoms, including persistent diarrhea, abdominal pain, nausea, and low-grade fever. *G. lamblia* infection

(giardiasis) can lead to symptoms such as acute or chronic diarrhea, malabsorption, flatulence, cramping, fatigue, and liver or pancreatic inflammations. *E. histolytica* infection (amebiasis) can cause symptoms like diarrhea, abdominal pain, fever, sepsis, liver abscesses, and skin lesions. *C. parvum* infection (cryptosporidiosis) can result in mild-to-acute diarrhea, vomiting, malabsorption, dehydration, weight loss, and biliary and respiratory involvement in immunodeficient individuals (Hemphill et al., 2019). These symptoms can vary in severity and duration depending on the specific protozoan pathogen involved and the individual's immune status. It is important to diagnose and treat these infections promptly to alleviate symptoms and prevent complications (Hemphill et al., 2019). Furthermore, *Blastocystis* sp. infection (Blastocystosis) in humans can cause a range of symptoms, including gastrointestinal issues such as diarrhea, abdominal pain, bloating, and flatulence. Other symptoms may include nausea, vomiting, fatigue, and weight loss (Shams et al., 2021).

Human infection with intestinal protozoan parasites usually occurs through ingestion of cysts/oocysts contaminating food or drinking water, including fresh vegetables. Current estimates indicate a global prevalence of approximately 20% for intestinal protozoan contamination in vegetables (Badri et al., 2022). The potential for intestinal protozoan contamination in fresh vegetables poses a significant public health concern due to various transmission pathways. These include fecal contamination of soil and water sources from human and animal waste, and the use of manure during irrigation. Additionally, insufficient hygiene practices throughout the vegetable production, processing, marketing, and consumer preparation chain further exacerbate the risk of contamination (Badri et al., 2022). While fresh vegetables offer an abundance of health benefits, neglecting proper hygiene, particularly concerning thorough washing and adequate cooking, can lead to the ingestion of these parasites and subsequent intestinal protozoan infections. These infections can manifest in various forms, including persistent diarrhea, abdominal cramps, and vomiting, posing a threat to individual health and well-being.

Surveys in Thailand have revealed the presence of pathogenic protozoa in many fresh vegetable types, including kitchen mint, lettuce, coriander, nappa cabbage, Chinese kale, cabbage, celery, leek, culantro, and water spinach. This contamination has also been reported for *Cyclospora cayentanensis*, *G. lamblia*, *Blastocystis* sp., *E. histolytica*, and *C. parvum* in Pathum Thani, Nakhon Nayok, and Payao Provinces, with a prevalence ranging from 6.3 to 57.0% (Krainara et al., 2023; Sripanompong et al., 2021; Tantiamornkul & Mataradchakul, 2019). In addition, previous studies in Thailand have demonstrated that protozoan contamination varies considerably among different types of fresh vegetables. Leafy vegetables, such as coriander, celery, and lettuce, are frequently reported to have relatively high contamination rates, ranging from 19% to 60%, 15% to 80%, and 2.9% to 90%, respectively. Similarly, kale has been reported with high contamination levels, ranging from 30% to 100% across different studies. Other vegetables, including water spinach, also showed variable contamination levels, ranging from 4.17% to 30%. In addition, hoary basil (36.7%) and spring onion (80%) have been reported with detectable contamination in certain studies. In contrast, yardlong bean consistently showed relatively low contamination rates, ranging from 2.5% to 3.3% (Krainara et al., 2023; Laoraksawong et al., 2025; Poochada et al., 2024; Sripanompong et al., 2021; Tantiamornkul & Mataradchakul, 2019).

Previous research on intestinal protozoan contamination in Thai fresh vegetables has focused entirely on market-sourced samples. However, a critical knowledge gap exists regarding contamination in vegetables cultivated in cultivation plots. This gap is particularly evident in Chachoengsao Province, where no studies have investigated protozoa in locally cultivated vegetables. Notably, reports of intestinal protozoa infections persist in

this region, with a prevalence ranging from 1.8 to 13.8% among schoolchildren and residents (Suntaravitun & Dokmaikaw, 2017; Suntaravitun & Dokmaikaw, 2018). This persistent burden underscores the need to investigate potential sources or reservoirs of infection within the local environment.

Therefore, our research focuses on investigating the prevalence of intestinal protozoa contamination in nine types of commonly eaten raw vegetables obtained from cultivation plots in Bang Khla District, Chachoengsao Province, Thailand. These vegetables include coriander, lettuce, celery, spring onion, hoary basil, kale, water spinach, sweet basil, and yardlong bean. The findings of this investigation will provide insights into the present scenario of protozoal outbreaks in fresh vegetables. Furthermore, the information obtained from this study will be essential for developing preventive strategies to stop the spread of these infections among the community.

Materials and Methods

Study Area

This study was conducted during April–December 2023 in nine vegetable farms across Bang Khla District, Chachoengsao Province, Thailand. Situated approximately 100 km east of Bangkok, the district covers 236.16 km² within the geographical coordinates of 13°43'42" N to 101°12'30" E. The region has a tropical climate, with an average annual temperature of 29.6°C and annual rainfall of approximately 1206.7 mm.

The selected farms represented typical small-scale vegetable cultivation systems in the study area. All farms were open-field systems using soil-based cultivation. Soil characteristics varied across farms, predominantly comprising loamy and sandy loam soils. Irrigation water was obtained from both groundwater (wells) and natural surface water sources such as canals and ponds. Fertilization practices included both chemical fertilizers and organic inputs, particularly animal manure. In addition, domestic and farm animals, including poultry, dogs, and cattle, were observed in or near some cultivation areas. These environmental and agricultural factors may influence protozoan contamination levels in fresh vegetables. The selected farms were distributed across multiple sub-districts within Bang Khla District where vegetable cultivation was present. Sub-districts without vegetable farming activities were excluded, as they were not relevant to the study objectives.

Sample Collection

This study was conducted as a cross-sectional survey of protozoan contamination in fresh vegetables at the farm level. The sample size (n) was determined using the formula:

$$n = \frac{Z^2 p(1-p)}{e^2}$$

where:

n = the sample size,

Z = the critical value associated with a 95% confidence level (set at 1.96 for a 95% confidence interval),

e = the margin of error, set at 0.05,

p = the probability of success, set at 0.60, reflecting the likelihood of detecting an occurrence. This was informed by Niamnuy et al. (2022), who reported that 60% of fresh vegetables in Thailand harbored contaminants.

By substituting $Z = 1.96$, $p = 0.60$, and $e = 0.05$ into the formula, the calculated sample size was 369. Quota sampling was used to ensure equal representation of each vegetable type, with 41 samples collected per

type. This approach may introduce selection bias, as samples were not selected using a probability-based sampling method. To improve representativeness, samples were collected from multiple locations within each cultivation plot. The selected vegetable varieties included coriander (*Coriandrum sativum*), lettuce (*Lactuca sativa*), celery (*Apium graveolens*), spring onion (*Allium cepa*), hoary basil (*Ocimum canum*), kale (*Brassica oleracea*), water spinach (*Ipomoea aquatica* Forsk.), sweet basil (*Ocimum basilicum*), and yardlong bean (*Vigna unguiculata*) (Fig. 1). The selection of vegetable types was based on their common consumption in raw form, widespread availability in the study area, and their potential risk for protozoan contamination, particularly for leafy vegetables.

A total of 369 fresh vegetable samples were collected from cultivation plots. Quota sampling determined the number of samples per vegetable type. Sample collection was conducted during the morning between 08:00 and 09:00 to minimize variation in environmental conditions. Vegetable samples were collected from multiple farms based on the availability of each vegetable type at the time of sampling. Not all vegetable types were present in every farm, reflecting differences in local cultivation practices. The number of samples collected from each farm and plot was not fixed but depended on the availability of each vegetable type at the time of sampling. Samples were collected from different locations within each plot to ensure spatial distribution and reduce location-specific bias. Sampling was conducted across different areas within each cultivation plot to ensure adequate spatial representation. After root portions were removed, samples were placed in sterile plastic bags, and transported to the Science and Technology Laboratory of Rajabhat Rajanagarindra University in Chachoengsao Province for subsequent analysis of intestinal protozoa contamination. All samples were processed within 2 hours of collection to preserve sample integrity.



Figure 1 Nine types of fresh vegetable samples were randomly collected from cultivation plots, including (1) coriander (*Coriandrum sativum*), (2) lettuce (*Lactuca sativa*), (3) celery (*Apium graveolens*), (4) spring onion (*Allium cepa*), (5) hoary basil (*Ocimum canum*), (6) kale (*Brassica oleracea*), (7) water spinach (*Ipomoea aquatica* Forsk.), (8) sweet basil (*Ocimum basilicum*), and (9) yardlong bean (*Vigna unguiculata*)

Laboratory diagnosis

Fresh vegetable samples were examined for intestinal protozoa using a modified sedimentation technique inspired by the methods outlined in Niamnuy et al. (2022) and Matosinhos et al. (2016). Our protocol involved aseptically chopping each ~200 g vegetable sample using dedicated knives and cutting boards. The chopped material was then transferred to individual new plastic bags containing 1000 ml of a detergent solution (1% sodium dodecyl sulfate, 0.1% Tween 80) and shaken at 200 rpm for 10 min to facilitate protozoan detachment from plant material. The wash water, potentially containing dislodged protozoa, was subsequently filtered through sterile gauze and collected in a beaker for further analysis. After a 2 h sedimentation period, the supernatant was removed, and the resulting sediment (approximately 10 ml) was transferred to centrifuge tubes with a capacity of 15 ml. The samples then underwent centrifugation at 1,500 rpm (327 x g) for 5 minutes. Following this centrifugation, the supernatant was carefully decanted, and the remaining material was resuspended in 0.85% NaCl to achieve a final volume of 15 ml. To further purify the sediment and potentially isolate any remaining parasites, the mixture was subjected to a second centrifugation at 1,500 rpm (327 x g) for 5 min. The supernatant was discarded after the final centrifugation. The sediment was resuspended using a sterile Pasteur pipette, fixed in 10% formaldehyde for preservation, stained with 1% Lugol's solution, and observed under a microscope for protozoan identification. The oocysts of coccidian species were identified using a modified acid-fast staining method following the procedures described by Garcia (2007) and Garcia et al. (2017). Each sample was examined in triplicate, and slide readings were independently verified by a second observer to ensure accuracy and consistency. For analytical classification, samples were categorized as mono-contamination when a single protozoan species was detected and as poly-contamination when two or more species were identified. Microscopic examination cannot differentiate between *Entamoeba histolytica* and *E. dispar*, as they are morphologically indistinguishable; therefore, identification was based on morphology only. Molecular methods such as polymerase chain reaction (PCR) are required for accurate differentiation.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, Version 23 (IBM SPSS Statistics for Windows, Version 23.0; Armonk, New York: IBM Corp). Intestinal protozoan prevalence in fresh vegetables is presented as frequency and percentage. To assess variations in the rates of intestinal protozoan contamination among different types of vegetables, the Chi-square (χ^2) test was applied. A significance level of $p < 0.05$ was considered statistically significant.

Results

This study investigated the prevalence and distribution of protozoan contamination in fresh vegetables. Contamination with cysts and oocysts from four protozoan species was detected among the examined fresh vegetable samples. Overall, the prevalence rates were as follows: *Cryptosporidium* spp. (44/369; 11.9%), *Entamoeba histolytica*/*E. dispar* (28/369; 7.6%), *Blastocystis* sp. (13/369; 3.5%), and *Giardia* spp. (7/369; 1.9%). A statistically significant difference in protozoan contamination was observed among different types of fresh vegetables ($\chi^2 = 36.952$, $p = 0.00001$).

When stratified by vegetable type, the distribution of contamination varied considerably across the nine vegetable types examined. Water spinach showed the highest contamination rate (29/41; 70.7%), followed by

coriander (15/41; 36.6%) and lettuce (14/41; 34.1%). Celery and kale each showed a contamination rate of (9/41; 22.0%). Hoary basil and sweet basil demonstrated lower contamination rates of (5/41; 12.2%) each, while spring onion showed a rate of (4/41; 9.8%). In contrast, yardlong bean exhibited the lowest contamination rate among all examined vegetables (2/41; 4.9%) (Table 1).

Table 1 Prevalence and distribution of protozoan contamination in fresh vegetables from nine vegetable farms in Bang Khla District, Chachoengsao Province, Thailand

Vegetable type	No. of samples	Types of protozoan contamination (%)		Total No. samples contaminate d (%)	Protozoa genera				χ^2	p- value
		Mono-contamination (n, %)	Poly-contamination (n, %)		<i>Bl</i>	<i>Crypto</i>	<i>Eh/Ed</i>	<i>Gi</i>		
Coriander	41	9 (22.0)	6 (14.6)	15 (36.6)	2	6	4	3	36.95	0.00001
Yardlong bean	41	2 (4.9)	0	2 (4.9)	0	1	1	0	2	^a
Celery	41	7 (17.1)	2 (4.9)	9 (22.0)	1	5	3	0		
Spring onion	41	3 (7.3)	1 (2.4)	4 (9.8)	0	2	1	1		
Hoary basil	41	4 (9.8)	1 (2.4)	5 (12.2)	1	2	1	1		
Kale	41	6 (14.6)	3 (7.3)	9 (22.0)	1	3	4	1		
Lettuce	41	8 (19.5)	6 (14.6)	14 (34.1)	3	8	3	0		
Water spinach	41	17 (41.5)	12 (29.3)	29 (70.7)	4	15	9	1		
Sweet basil	41	5 (12.2)	0	5 (12.2)	1	2	2	0		
Total (%)	369	61 (16.5)	31 (8.4)	92 (24.9)	13(3.5)	44(11.9)	28(7.6)	7(1.9)		

Remark: *Bl* = *Blastocystis* sp., *Crypto* = *Cryptosporidium* spp., *Eh/Ed* = *E. histolytica*/*E. dispar*, *Gi* = *Giardia* spp. ^a p-value considered significant at $p < 0.05$.

Discussion

The prevalence of protozoal contamination in fresh vegetables was determined to be 24.9% (92/369) using the sedimentation technique for detecting contamination. This percentage is higher than the prevalence reported in a study conducted in the Nakhon Nayok Provinces of Thailand, which found a pathogenic protozoan prevalence of 14.1% (53/375) (Krainara et al., 2023). The results of our study were compared with the findings of past research conducted by Tantiamornkul & Mataradchakul (2019) in the Phayao Province of Thailand. Their research identified that vegetable samples were contaminated with at least one type of protozoa, with a prevalence of 57.0% (57/100) as determined through the use of the PCR technique. Additionally, the prevalence observed in this study was lower than that reported in Egypt, where a protozoan contamination rate of 74.5% (298/400) was reported (Yahia et al., 2023), as well as in Syria with a rate of 27.3% (35/128) (Al Nahhas & Aboualchamat, 2020), and Ethiopia with a rate of 29.4% (113/384) (Alemu et al., 2020). Our results align most closely with those reported from another study in Ethiopia, which found a comparable prevalence of 22.8% (Asfaw et al., 2023). These variations may be explained by differences in detection methods, particularly the higher sensitivity of molecular techniques compared to microscopy, as well as environmental and agricultural factors influencing contamination levels.

Our study detected *Cryptosporidium* spp. in only 11.9% (44/369) of fresh vegetable samples, which is significantly lower than the 35.0% (35/100) contamination rate reported by Tantiamornkul & Mataradchakul (2019) in Phayao Province, northern Thailand. However, it is higher than the prevalence reported in Chonburi Province, eastern Thailand, where a contamination rate of 2.0% (2/100) was observed using combined

microscopy and PCR techniques (Sutthikornchai et al., 2025). Our findings align with the prevalence reported in Ghana at 11.1% (40/360) (Kudah et al., 2018). Nevertheless, they fall below those reported in Egypt at 16.5% (66/400) (Yahia et al., 2023) and Iran at 21.42% (3/14) (Taghipour et al., 2019). Conversely, our results are higher than those observed in Spain at 6.8% (4/59) (Moreno-Mesonero et al., 2023) and Ethiopia, with a prevalence range of 1.9 to 5.8% (Alemu et al., 2019; Alemu et al., 2020; Bekele et al., 2017). The presence of *Cryptosporidium* spp. in contaminated food, especially fresh vegetables, underscores the significance of adopting proper hygiene practices during harvesting, handling, and preparation of leafy greens and other produce. This highlights the importance of understanding regional variations in contamination rates and implementing appropriate measures to ensure food safety. *Cryptosporidium* spp. represents a major cause of mortality linked to waterborne gastrointestinal diseases and constitutes one of the most significant global waterborne pathogens. The detection of *Cryptosporidium* spp. in water presents challenges due to their low prevalence and the intricate nature of the water matrix (Hassan et al., 2021). The study revealed a higher prevalence of *Cryptosporidium* spp. in water spinach compared to other vegetables. Given that water spinach is a floating aquatic plant, this discovery implies that the water used for irrigating water spinach may carry *Cryptosporidium* spp. contamination. There is also a risk that other vegetables may become contaminated if this water is subsequently used for irrigation elsewhere on the farm.

Our investigation revealed a prevalence of 7.6% (28/369) in fresh vegetables for *Entamoeba histolytica*/*E. dispar* cysts. This percentage exceeds the 0.5% (2/375) found exclusively in celery among various vegetables investigated in Nakhon Nayok Province, central Thailand, utilizing the sedimentation technique (Krainara et al., 2023). It is also greater than the prevalence reported in the Bangkok metropolitan region and surrounding areas, where *Entamoeba* spp. contamination was detected in iceberg lettuce at 0.11% (2/1800) using microscopy-based sedimentation methods (Laoraksawong et al., 2025). However, our results are lower than the 34.0% (34/100) reported in a previous study conducted in Phayao Province, northern Thailand, utilizing PCR techniques for detection (Tantiamornkul & Mataradchakul, 2019). Notably, kitchen mint and nappa cabbage emerged as the most commonly contaminated vegetables in that study, while Thai water convolvulus (water spinach) was not found to be contaminated.

These variations in prevalence rates may be attributed to differences in vegetable types as well as variations in detection methodologies. In particular, molecular techniques such as polymerase chain reaction (PCR) are generally recognized for their higher sensitivity and specificity compared to conventional microscopy. This may explain the higher prevalence reported in studies using PCR-based methods, whereas microscopy-based approaches, as employed in the present study, may underestimate the true prevalence due to lower detection sensitivity. In addition, variations in environmental conditions and contamination sources, including human, animal, and environmental reservoirs, may further contribute to differences in protozoan prevalence across geographical locations.

Compared to research reports from other countries, the prevalence of contamination in this study was lower than that in Egypt, which reported a rate of 13.7% (55/400) (Yahia et al., 2023). In Ethiopia, the prevalence ranged from 8.4 to 12.8% according to various studies (Alemu et al., 2020; Asfaw et al., 2023; Bekele & Shumbej, 2019; Gemechu et al., 2023). Nevertheless, it was higher than the prevalence observed in Mozambique at 3.4% (11/321) (Salamandane et al., 2021). Species differentiation between *E. histolytica* and *E. dispar* is not feasible through microscopy alone. Therefore, molecular biology techniques are essential to

accurately distinguish between these two species, enabling a more comprehensive understanding of their true distribution and facilitating appropriate disease prevention and control strategies.

The present study identified a prevalence of 3.5% (13/369) for *Blastocystis* sp. contamination in fresh vegetables using the sedimentation technique. Among the vegetables examined, water spinach exhibited the highest contamination rate. This finding is comparable to the 3.2% (12/375) prevalence reported by Krainara et al. (2023) in Nakhon Nayok Province, central Thailand, where celery showed the highest contamination rate, followed by leek and lettuce. However, it is important to note that water spinach was not included in their study; therefore, caution should be exercised when making direct comparisons due to differences in vegetable profiles. Similarly, a study conducted in the Bangkok metropolitan region and surrounding areas reported a prevalence of 1.56% (28/1800), with contamination detected in vegetables such as tomato, cabbage, cucumber, culantro, and celery, using microscopy-based sedimentation methods (Laoraksawong et al., 2025). In contrast, a much higher prevalence of 24.7% (74/300) was reported in Khon Kaen Province, northeastern Thailand, where contamination was observed in a wide range of vegetables, including peppermint, celery, Thai basil, coriander, Chinese kale, cabbage, lettuce, Chinese cabbage, and yardlong bean, also using microscopic examination (Poochada et al., 2024). Furthermore, Jinatham et al. (2023) reported an even higher prevalence of 45% (9/20) in Chiang Rai Province, northern Thailand, using qPCR techniques, with contamination detected in multiple vegetables, including beetroot, cabbage, carrot, Chinese cabbage, galangal, kale, lemongrass, tomato, and Chinese morning glory root. The lower prevalence observed in the present study may be attributed to the use of microscopy, which has lower sensitivity and specificity compared to molecular techniques such as qPCR. Nevertheless, the findings of this study, together with previous reports from different regions of Thailand, indicate that *Blastocystis* sp. remains a common contaminant of fresh vegetables, although the reported prevalence varies considerably depending on geographic location, vegetable types, and detection methods.

In comparison with previous studies conducted in other countries, the prevalence of *Blastocystis* sp. contamination in fresh vegetables in this study was found to be similar to that of Iran at 4.2% (10/240) (Isazadeh et al., 2020). Nevertheless, this study reported a lower prevalence than that observed in Egypt at 39.5% (34/86) (El-Sayed et al., 2023), Syria at 10.1% (13/128) (Al Nahhas & Aboulchamat, 2020), Mexico at 9.2% (37/400) (Morales-Figueroa et al., 2021), Lebanon at 8.7% (26/300) (El Safadi et al., 2023), and Egypt at 7.0% (28/400) (Yahia et al., 2023). Additionally, the prevalence in this study was higher than that reported in Mozambique at 1.3% (4/321) (Salamandane et al., 2021) and Spain at 1.8% (2/110) (Moreno-Mesonero et al., 2023). The variations in prevalence of intestinal protozoan parasites across different countries can be attributed to various factors associated with each geographical location, such as climate, water sources, sanitation infrastructure, and agricultural practices.

The prevalence of *Giardia* spp. contamination in fresh vegetables was 1.9% (7/369) in this study, determined using the sedimentation technique. Notably, water spinach showed a significantly higher contamination rate compared to other vegetables. Our findings are lower than those reported in several previous studies in Thailand. For example, Krainara et al. (2023) reported a prevalence of 4.0% (14/375) in Nakhon Nayok Province using the sedimentation technique, with the highest contamination observed in celery and leek, followed by coriander, peppermint, and lettuce. Similarly, Sripanompong et al. (2021) reported a prevalence of 6.2% (6/96) using nested PCR in Pathum Thani Province, where contamination was detected in napa cabbage, cabbage, and lettuce. It is important to note that neither of these studies included water spinach for parasite

detection. A higher prevalence of 13.0% (13/100) was also reported in Phayao Province using PCR techniques (Tantiamornkul & Mataradchakul, 2019), with contamination identified in kitchen mint, lettuce, coriander, napa cabbage, Chinese kale, cabbage, spring onion, and water spinach.

In contrast, a lower prevalence of 0.28% (5/1800) was reported in the Bangkok metropolitan region and surrounding areas using microscopy-based methods, with contamination detected in coriander and iceberg lettuce (Laoraksawong et al., 2025). In addition, a prevalence of 2.0% (2/100) was reported in Chonburi Province, eastern Thailand, using combined microscopy and PCR techniques (Sutthikornchai et al., 2025), which is comparable to the findings of the present study. These findings suggest that *Giardia* spp. contamination varies across Thailand and may be influenced by differences in geographic location, vegetable types, and detection methods.

A comparison with previous studies conducted in other countries found that the prevalence of *Giardia* spp. contamination in fresh vegetables in this study was similar to that of Spain at 1.7% (1/59) (Moreno-Mesonero et al., 2023), Lebanon at 1.0% (3/300) (El Safadi et al., 2023), and Saudi Arabia at 1.0% (4/400) (Gabre & Shakir, 2016). It was also higher than that of Tunisia at 0.4% (1/240) (M'rad et al., 2020). Nevertheless, it was lower than that of Egypt at 26.0% (104/400) (Yahia et al., 2023), Zambia at 24.2% (23/95) (Nyirenda et al., 2021), Ethiopia at 6.4–13.3% (Alemu et al., 2019; Alemu et al., 2020; Asfaw et al., 2023; Bekele et al., 2017; Bekele & Shumbej, 2019; Gemechu et al., 2023), Syria at 13.3% (17/128) (Al Nahhas & Aboualchamat, 2020), Iraq at 10.6% (24/225) (Mirzaei et al., 2021), Iran at 9.2% (29/316) (Balarak et al., 2016), Jordan at 6.8% (9/133) (Ismail, 2016), Mozambique at 3.7% (12/321) (Salamandane et al., 2021), Iran at 3.7% (9/240) (Isazadeh et al., 2020), and Sudan at 3.0% (8/260) (Mohamed et al., 2016). These international comparisons highlight the influence of regional factors on *Giardia* spp. contamination in fresh produce, emphasizing the need for region-specific prevention and control strategies.

The highest prevalence of protozoan contamination was found in water spinach at 70.7% (29/41), exceeding the 30% prevalence reported in a previous study from Phayao Province, northern Thailand, by Tantiamornkul & Mataradchakul (2019) using PCR. The markedly higher contamination observed in water spinach further supports the role of irrigation water and cultivation conditions as key determinants of protozoan contamination. This difference might be attributable to geographic location or the source of the water spinach. In this study, water spinach was collected directly from the water cultivation plot of the vegetable garden, potentially leading to higher contamination compared to market-bought water spinach, which undergoes washing before sale to reduce contamination. Water spinach, a semi-aquatic plant widely cultivated as a vegetable in Southeast Asian countries, thrives in both water and moist soil. It is commonly found in diverse habitats such as rivers, canals, rice fields, ponds, lakes, kitchen gardens, vegetable patches, roadsides, and public water bodies. The presence of protozoan contamination in water spinach suggests that the water used to irrigate it is also contaminated. Eating raw water spinach collected from natural water bodies may pose a risk of human infection with protozoan pathogens, especially those that cause gastrointestinal diseases. Contaminated water used for irrigation can spread the parasites to other vegetables. Ensuring vegetable safety requires treating and rigorously testing irrigation water. However, contamination can also originate within the garden itself. Therefore, future studies should routinely collect and analyze cultivation water samples from vegetable gardens for pathogenic protozoan contamination. This proactive approach has the potential to identify the source of contamination,

guiding the development of targeted prevention and control measures. Ultimately, it plays a crucial role in safeguarding both public health and agricultural sustainability by promoting safe food production practices and protecting consumer health.

The variability in protozoan contamination across diverse regions can be attributed to a complex interplay of multiple interrelated factors. These include geographic variation in pathogen prevalence, environmental conditions influencing protozoan survival, the characteristics of different vegetable types, and the presence of animal reservoirs within cultivation areas. In addition, agricultural practices, including cultivation methods, the use of animal manure, and irrigation water quality, play a critical role in shaping contamination patterns. Importantly, these factors do not act independently but are interconnected within environmental transmission pathways that facilitate the movement of protozoan cysts and oocysts from environmental reservoirs, including contaminated water, soil, and animal sources, onto vegetable surfaces. This interconnected pathway highlights how environmental exposure, agricultural practices, and biological reservoirs collectively contribute to the contamination of fresh produce, particularly in settings where hygiene management and water quality control are insufficient.

This study has several limitations that should be considered. The use of quota sampling may introduce selection bias, as the samples may not fully represent all vegetable cultivation in the study area. Additionally, variation in vegetable availability across farms and the sampling design limited the ability to perform balanced farm-level comparisons. The use of microscopy may have underestimated the true prevalence due to its lower sensitivity compared to molecular techniques. Environmental samples, such as irrigation water and soil, were not analyzed, limiting the ability to identify potential sources of contamination. Confidence intervals were not calculated and should be reported in future studies to enhance statistical interpretation. Future studies employing probability-based sampling methods and incorporating molecular and environmental analyses are recommended to improve representativeness and better understand transmission pathways.

Conclusion and Suggestions

This research represents the first comprehensive study of intestinal protozoan contamination in fresh vegetables harvested from cultivation areas in Thailand. Notably, water spinach exhibited markedly higher levels of contamination compared to other vegetables examined. The markedly high contamination observed in this semi-aquatic plant strongly suggests that irrigation water and environmental factors play a critical role in protozoan contamination. These findings highlight the importance of both consumer-level practices, such as proper washing and food handling, and upstream environmental control measures, including irrigation water quality management and improved agricultural sanitation. Inadequate hygiene practices may further increase the risk of protozoan transmission.

This study provides important insight into contamination pathways at the cultivation level, which are often overlooked in studies focusing on market-based samples. The findings have important public health implications, emphasizing the need to strengthen food safety education, improve sanitation in cultivation environments, and promote safe water use in agriculture to reduce the burden of foodborne protozoan infections. Future research should incorporate larger sample sizes, expanded geographic coverage, and more sensitive diagnostic techniques,

such as molecular methods, as well as environmental sampling of irrigation water and soil to better understand transmission pathways.

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Conflict of Interests

All authors declare that they have no conflicts of interest.

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Declaration of Generative AI and AI-assisted Technologies

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, sentence refinement, and improvement of academic clarity. The tool was also used on a limited basis to help improve the organization and presentation of the manuscript. Following its use, the authors carefully reviewed, revised, and validated all content to ensure that it accurately reflects their work. The authors accept full responsibility for the accuracy, originality, and final version of the manuscript.

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