PREVALENCE OF HELMINTH CONTAMINATION OF FRESH VEGETABLES FROM CULTIVATION PLOTS IN BANG KHLA DISTRICT, CHACHOENGSAO PROVINCE, THAILAND

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Abstract. Intestinal helminthic parasites pose a serious threat to public health. The prevalence of helminth contamination in fresh vegetables collected from nine farms in Chachoengsao Province, Thailand was investigated. Sedimentation technique was used to screen 359 fresh vegetable samples for helminths following washing with a detergent solution. The overall 38% prevalence of helminth contamination in the study highlighted the widespread nature of this issue. Of the nine vegetables, 63% of lettuce had the highest proportion of contamination, followed by 51% for coriander, 44% for celery, 41% for sweet basil, 37% for hoary basil, 34% for spring onion, 32% for kale, 24% for water spinach, and 15% for yearlong beans. Six genera of helminths were identified, present in both egg and larval stages, namely, Ancylostomatoidea (hookworm), *Ascaris* spp, *Strongyloides* spp, *Taenia* spp, *Trichuris* spp, and *Toxocara* spp. Of note, hookworms were the most prevalent helminth in egg (8%) and larval (15%) stages. Contamination by the helminths significantly differed among the nine vegetables (p-value = 0.0003, using the Chi-square test). The high prevalence of helminth contamination in fresh vegetables obtained from local farms in this province raises concerns about potential transmission to humans. This finding underscores the critical importance of stringent hygiene practices, such as thorough washing, disinfection and proper cooking, to ensure vegetables are devoid of parasitic and pathogenic contaminants. Education and communication of these measures will assist public awareness of proper food handling practices to ensure vegetables are safe for human consumption.

Keywords: foodborne parasite, fresh vegetable, helminth contamination, intestinal parasite infection

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INTRODUCTION

Helminthic parasite infections remain a significant public health concern, particularly in tropical and subtropical regions across various countries. These neglected diseases affect marginalized populations in low- and middle-income nations (Montresor *et al*, 2020). Soil-transmitted helminths, a notable group among these infections, pose a risk to more than one in four people globally. The group includes species like *Ancylostoma duodenale* and *Necator americanus* (hookworm), *Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Trichuris trichiura* (Jourdan *et al*, 2018). Affected individuals have varying degrees of illness, ranging from mild cases often showing no symptoms when few parasites are present to severe infections leading to a range of symptoms, including gastrointestinal complications, nutritional deficiencies, weakness, fever, and compromised physical growth and development (WHO, 2023).

The prevalence of soil-transmitted helminths is closely linked to prevailing socioeconomic conditions, where inadequate sanitation and poverty contribute to an escalating prevalence of infections. Transmission of these helminths primarily occurs through exposure to a contaminated environment hosting parasitic eggs, commonly happening when people ingest water, soil, food, or vegetables tainted with human or animal feces. Additionally, the risk of infection rises when untreated wastewater or natural water sources become contaminated with parasites, which often arise in agricultural practices, such as animal husbandry or vegetable cultivation, the latter involving the use of animal manure as fertilizer, potentially facilitating the spread of pathogens among the plants -- consequently, this practice results in the dissemination of infectious agents to consumers who consume fresh vegetables. Soil-transmitted helminth eggs of *Ascaris* spp, hookworm, *Taenia* spp, *Toxocara*

spp, and *Trichuris* spp, have been reported in soil samples collected from vegetable plots in both organic and conventional farms (Paller and Babia-Abion, 2019). Therefore, vegetables cultivated in these farms can become contaminated with helminths that are transmitted to consumers.

Fresh vegetables are recognized to provide essential nutrients crucial for overall good health, including a diverse range of antioxidants, electrolytes, minerals, phytochemicals, and vitamins (Slavin and Lloyd, 2012). However, there is a potential risk of contracting diseases when consuming fresh vegetables containing pathogens from soil and water used during cultivation. Recent studies conducted in Thailand have highlighted the high prevalence (35-88%) of helminth contaminations in various fresh vegetables collected from markets across multiple provinces (Katasrila and Laoju, 2018; Punsawad *et al*, 2019; Pednog and Toonsakool, 2020; Niamnuy *et al*, 2022). However, a significant gap in research remains regarding the presence of helminths in vegetables freshly harvested directly from the cultivation plots, including Chachoengsao Province, Thailand. Despite reports of repeated outbreaks of parasite infection among residents (Suntaravitun and Dokmaikaw, 2017; Suntaravitun and Dokmaikaw, 2018), suggesting a prevalent issue with parasitic infections in this region, no investigation has been conducted to ascertain the presence of helminth parasites specifically in fresh vegetables.

Hence, our research aims to explore the prevalence of helminth contamination in fresh vegetables directly obtained from fields in Chachoengsao Province. Knowledge of parasitic contamination in vegetables directly harvested from farm fields is crucial for developing strategies to prevent the transmission of parasite-infested vegetables from farms to fresh produce markets, thereby ensuring the safety of this important consumable commodity for the benefit of both consumers and producers.

MATERIALS AND METHODS

Study area

Vegetable samples were collected from nine farms situated in Bang Khla District, Chachoengsao Province, Thailand between April and December 2023. The study area covers an area of 236.16 km^2 located

about 100 km east of Bangkok. Geographically, it extends from 13° 43′ 42″ north latitude to 101° 12′ 30″ east longitude (Fig 1). The region receives an annual rainfall of 1,206.7 mm and has an average temperature of 29.6°C throughout the year. All test farms practiced open farming systems with soil cultivation utilizing a combination of wells and natural water sources for irrigation. Fertilization practices of the farms include both chemical fertilizers and animal manure. Additionally, the presence of cattle, dogs and poultry was observed near the growing areas on some farms.

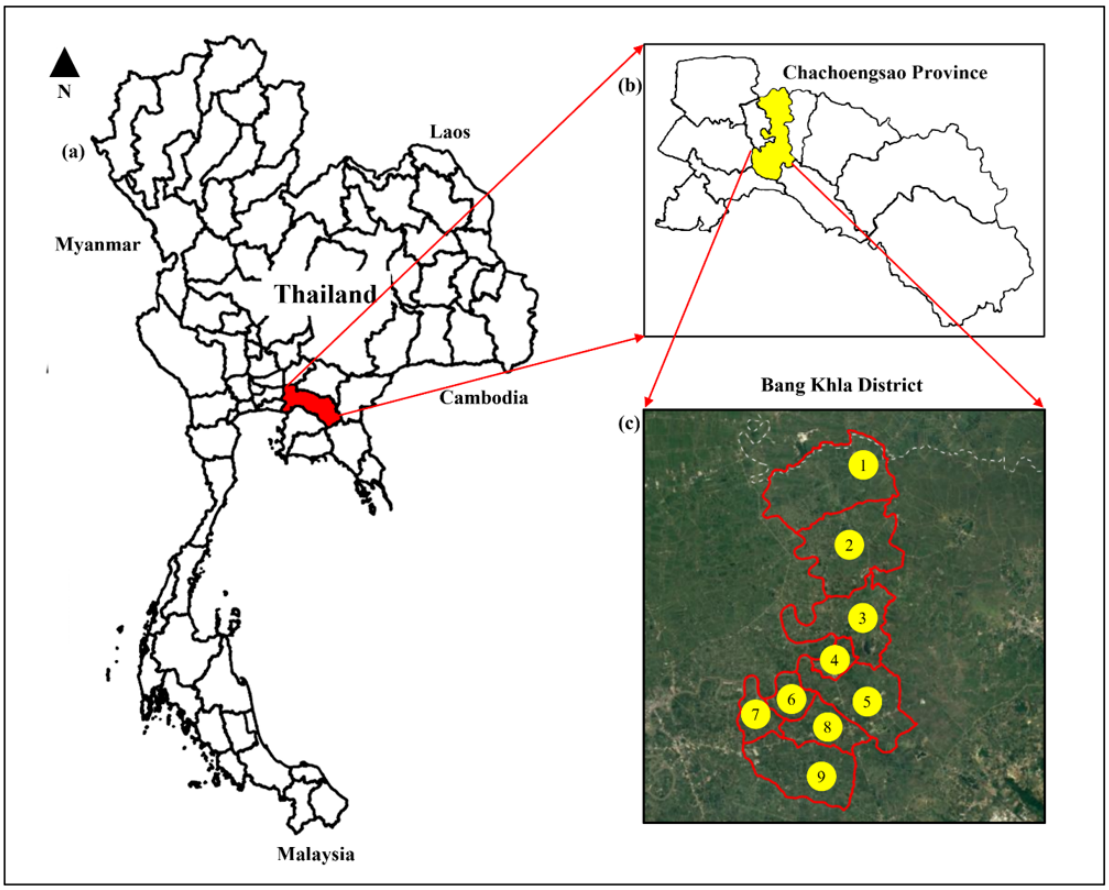

Fig 1 - Map showing the locations of the nine vegetable farms, Bang Khla District, Chachoengsao Province, Thailand

Sample collection

The sample size was calculated using the following equation:

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

where n = sample size

Z = critical value linked to a 95% confidence level (set at 1.96)

e = margin of error (set at 0.05), and

p = the probability of success (set at 0.60)

This determination aligns with the findings from a previous study by Niamnuy *et al* (2022), which reported a 60% prevalence of contaminants in fresh vegetables in Thailand. When the values Z = 1.96, p = 0.60 and e = 0.05 were used in the equation, the resulting calculated sample size (n) was 369. A total of nine distinct types of fresh vegetables were acquired using quota sampling, with 41 samples collected for each vegetable type. The collection included celery (*Apium graveolens*), coriander (*Coriandrum sativum*), hoary basil (*Ocimum canum*), kale (*Brassica oleracea*), lettuce (*Lactuca sativa*), spring onion (*Allium cepa*), sweet basil (*Ocimum basilicum*), water spinach (*Ipomoea aquatica* Forsk), and yardlong bean (*Vigna unguiculata*) (Fig 2). These nine varieties of fresh vegetable samples were randomly collected from cultivation plots, ensuring a comprehensive representation by selecting samples from diverse areas within the plots. The collection process comprised meticulous excision of root proportion from the gathered vegetables, and these samples were carefully stored in pathogen-free plastic bags. Subsequently, all samples were stored in an ice box at approximately 4°C and transported to the Science and Technology Laboratory, Rajabhat Rajanagarindra University in Chachoengsao Province to identify helminth contamination, with a transit time of approximately 2 hours.

Laboratory helminth identification

Identification of helminths in fresh vegetables used a modified sedimentation method (Matosinhos *et al*, 2016; Niamnuy *et al*, 2022). Each vegetable sample (~200 g) (n = 3) was cut into small pieces using a sterile knife, placed in a plastic bag containing 1.0 l of detergent solution (1% (w/v) sodium dodecyl sulfate and 0.1% (v/v) Tween 80), shaken at 200 round per minute (rpm) for 10 minutes, and

Fig 2 - The nine types of fresh vegetable samples collected from cultivation plots (1): Coriander (*Coriandrum sativum*); (2): Lettuce (*Lactuca sativa*); (3): Celery (*Apium graveolens*); (4): Leek (*Allium porrum*); (5): Hoary basil (*Ocimum canum*); (6): Kale (*Brassica oleracea*); (7): Water spinach (*Ipomoea aquatica Forsk*); (8): Sweet basil (*Ocimum basilicum*); (9): Yardlong bean (*Vigna unguiculata*)

filtered through a sterile fine mesh gauze (600-700 μm mesh size). The filtrate was allowed to stand for 2 hours and supernatant discarded. To the sediment was added approximately 5-10 ml of 0.85% (w/v) NaCl, centrifuged at 327g for 5 minutes, supernatant discarded and pellet resuspended in 15 ml of 0.85% (w/v) NaCl. Following centrifugation as described above, supernatant was decanted, pellet homogenized and treated with 10% (v/v) formaldehyde. The samples were stained with 1% (v/v) Lugol's solution (Sigma-Aldrich, St Louis, MO), and observed under a light microscope (100x and 400x magnifications) to identify helminth eggs and larval stages according to the keys of Garcia (2007) and Taylor *et al* (2016).

Statistical analysis

The prevalence of helminths in fresh vegetables is expressed as percentage. The Chi-square test was used to compare the differences in the prevalence of helminth contamination among the nine different types of vegetables. A p-value <0.05 is considered statistically significant. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows, Version 23 (IBM Corp, Armonk, NY).

RESULTS

The results showed that fresh vegetables collected from nine farms in Bang Khla District, Chachoengsao Province, Thailand, were contaminated with helminths covering six genera, with five identified at the egg stage and two at the larval stage (Table 1). The overall prevalence of helminth contamination was 38%, with the highest prevalence being hookworm (8%) and the lowest *Toxocara* spp (1%) (Table 1). Lettuce had the highest frequency of helminth infestation (63%), followed by coriander (51%), then celery (44%), sweet basil (41%), hoary basil (37%), spring onion (34%), kale (32%), water spinach (24%), and yardlong bean (15%); the difference among the nine vegetable types is significantly different (χ^2 = 28.820, p-value = 0.0003; using a Chi-square test). Interestingly, the overall frequency of egg and larval contamination was equal (19%); however, only larvae of hookworm (15%) and *Strongyloides* spp (4%) were found, and eggs of the latter were not detected. Among the 369 collected vegetable samples, 115 (31%) were contaminated with one genus of helminths, 18 (5%) with two genera, and 7 (2%) with three genera.

Table 1

Prevalence of helminth contamination in fresh vegetables from nine cultivation plots, Bang Khla District, Chachoengsao Province, Thailand

Helminth species	Number of vegetable samples with parasites (41 samples from each vegetable type)									Total (369) *n* (%)
	Coriander	Yardlong bean	Celery	Spring onion	Hoary basil	Kale	Lettuce	Water spinach	Sweet basil	
Egg										70 (19)
Ascaris spp	2	2	2	1	0	2	3	5	4	21 (6)
Hookworm	3	2	3	2	4	2	5	3	6	30 (8)
Toxocara spp	0	0	0	0	0	2	0	1	1	4 (1)
Trichuris spp	0	2	0	1	3	0	1	1	1	9 (2)
Taenia spp	0	0	0	2	1	1	1	0	1	6 (1)
Larva										70 (19)
Hookworm	14	0	9	5	6	4	15	0	3	56 (15)
Strongyloides spp	2	0	4	3	1	2	1	0	1	14 (4)
Total, *n* (%)	21 (51)	6 (15)	18 (44)	14 (34)	15 (37)	13 (32)	26 (63)	10 (24)	17 (41)	140 (38)

DISCUSSION

The study showed that the overall prevalence of helminth contamination of nine types of fresh vegetables collected directly from nine cultivation plots in Bang Khla District, Chachoengsao Province, Thailand between April and December 2023, including both egg and larval stages, was 38%. Compared to prior research studies conducted in Thailand, our study's finding is in agreement with that (35%) of research conducted in Nakhon Si Thammarat Province, southern Thailand (Punsawad *et al*, 2019), but lower than that (50%) of a study in Phra Nakhon Si Ayutthaya Province, central region, (Jongkolnee and Songthamwat, 2015); and that (88%) of another study conducted in four regions of the country, namely, central (Bangkok and Pathum Thani Province), northern (Chiang Mai and Phitsanulok Provinces), northeastern (Khon Kaen and Nakhon Ratchasima Provinces), eastern (Chonburi and Rayong Provinces), and southern (Songkhla and Trang Provinces) (Pednog and Toonsakool, 2020).

In other countries, reports of helminth contamination of fresh vegetables were varied: in Ethiopia, the prevalence ranged from 19% (Alemu *et al*, 2020) to 49% (Asfaw *et al*, 2023), and in the Philippines, it was 24% (Verizon *et al*, 2019). Thus, the frequency of helminth contamination in fresh vegetables is influenced by various factors, like vegetable type, the status of presence of helminths in the cultivation soil and/or water, and the season and time of day during which fresh vegetable samples are collected (Salamandane *et al*, 2021; Osafo *et al*, 2022; Asfaw *et al*, 2023).

In our study, hookworm (both egg and larval stages) was the predominant helminth (15%) present in the fresh vegetables. Previous studies in Thailand, such as those by Pednog and Toonsakool (2020) reported a larval nematode contamination rate of 13%, while Punsawad *et al* (2019) reported a prevalence of hookworm eggs of 17%. In other countries, Ethiopia reported a prevalence of hookworm in fresh vegetables at 3% (Gemechu *et al*, 2023), Iran at 5% (Isazadeh *et al*, 2020), Iraq at 4% (Mirzaei *et al*, 2021), the Philippines at 2% (Alemu *et al*, 2019; Alemu *et al*, 2020) and 7% (Vizon *et al*, 2019), and Tunisia at 2% (M'rad *et al*, 2020).

Hookworm infection occurs when the larval stage penetrates the skin, typically through the hands and feet, leading to irritation and a

characteristic rash known as ground itch. Infections with *Ancylostoma duodenale* and *Necator americanus* can also occur through the ingestion of the larval stage, leading to penetration of the gastrointestinal tract (Loukas *et al*, 2016). Therefore, farmers cultivating vegetables in direct soil contact may be exposed to hookworms through the skin. Furthermore, consumers ingesting fresh vegetables contaminated with infective larvae of *A. duodenale* and *N. americanus* may be susceptible to infection.

Our findings of 4% prevalence for *Strongyloides* spp contamination of fresh vegetables contrasts with the previously reported prevalence of 11% in Nakhon Si Thammarat Province (Punsawad *et al*, 2019). Moreover, our study's prevalence rate is comparatively lower than that observed in other countries, such as Ethiopia at 13% (Alemu *et al*, 2020), Ghana at 36% (Kudah *et al*, 2018) and Saudi Arabia at 4% (Gabre and Shakir, 2016). However, a lower prevalence of *Strongyloides* spp contamination has been reported: in Lebanon at <1% (El Safadi *et al*, 2023), Mozambique at 1% (Salamandane *et al*, 2021), Sudan at 1% (Mohamed *et al*, 2016), and Syria at 1% (Al Nahhas and Aboualchamat, 2020). While we identified *Strongyloides* spp in several types of vegetables, it is of note that in Malaysia *Strongyloides* spp contamination was found only in *Centella asiatica* (pennywort or pegaga) (Yusof *et al*, 2017).

Strongyloides stercoralis is capable of percutaneous transmission akin to hookworms, and individuals contracting this parasitic infection may exhibit a range of symptoms, primarily gastrointestinal distress. However, immunocompromised subjects often experience severe diarrheal symptoms (Akanksha *et al*, 2023). The complexities involved in identifying *Strongyloides* spp in vegetable samples (AF White *et al*, 2019) underscore the critical necessity for tailored, region-specific strategies to comprehend and mitigate the potential risks associated with helminthic infections in diverse populations.

The high prevalence we observed of *Ascaris* spp contamination in fresh vegetables of 6% surpassed the previously reported rate of 3% in Nakhon Si Thammarat Province (Punsawad *et al*, 2019), but was lower than that (28%) in Samut Songkhram Province (Katasrila and Laoju, 2018) and that (9%) in Nakhon Nayok Province (Krainara *et al*, 2023). In other countries,

the prevalence of *Ascaris* spp contamination in fresh vegetables also revealed a wide range: Egypt at 51% (Yahia *et al*, 2023), Ethiopia at 1-21% (Bekele *et al*, 2017, Alemu *et al*, 2019; Bekele and Shumbej, 2019; Alemu *et al*, 2020), Iran at 5-24% (Balarak *et al*, 2016; Isazadeh *et al*, 2020), Jordan at 21% (Ismail, 2016), Lebanon at 4% (El Safadi *et al*, 2023), Saudi Arabia at 10% (Gabre and Shakir, 2016), Sudan at <1% (Mohamed *et al*, 2016), and the Philippines at 13% (Vizon *et al*, 2019). Similarly, the reported differences in *Ascaris* spp contamination rates may be attributed to environmental factors such as climate, soil composition, and agricultural practices. Of note, the thick eggshell of *Ascaris* spp provides exceptional resilience, allowing them to withstand harsh weather conditions, including freezing and intense heat, ensuring prolonged persistence in the environment (Wu *et al*, 2024).

Our finding of the prevalence of *Trichuris* spp contamination in fresh vegetables at 2% agrees with the previous study in Nakhon Si Thammarat Province (3%) (Punsawad *et al*, 2019). In other countries, the prevalence was reported in Egypt at 25% (Yahia *et al*, 2023), Ethiopia at <1-3% (Alemu *et al*, 2020; Gemechu *et al*, 2023), Iraq at 7% (Mirzaei *et al*, 2021), Lebanon at <1% (El Safadi *et al*, 2023), the Philippines at 2% (Vizon, 2019), and Tunisia at 1% (M'rad *et al*, 2020).

Trichuris trichiura exhibits pathogenicity in human hosts and successful transmission requires embryonation of eggs in the environment, but which does not occur at temperatures below freezing or above 37 °C (Else *et al*, 2020). Zoonotic infections have been reported for *T. suis* (from pig) and *T. vulpis* (from dog) (Mohd-Shaharuddin *et al*, 2019). However, infections from these parasites typically are attenuated and parasites rarely reach sexual maturity within the human host.

Our finding of the prevalence of *Taenia* spp in fresh vegetables at 2% contrasts with previous research conducted in various provinces of the country reporting an absence of contamination by this helminth (Punsawad *et al*, 2019; Pednog and Toonsakool, 2020). Low contamination rates (<1%) have been observed in Ethiopia (Gemechu *et al*, 2023), Syria (Al Nahhas and Aboualchamat, 2020), and the Philippines (Vizon *et al*, 2019). However, another survey in Ethiopia reported a prevalence

of 4% (Asfaw *et al*, 2023). A systematic review and meta-analysis in Iran from 2010 to 2020 on the prevalence of *Taenia* spp revealed egg contamination rates ranging from 0.9-30% in vegetable samples and from 0-43% in soil and water samples (Jansen *et al*, 2021).

Investigations of the survival of *Taenia* spp eggs under laboratory conditions revealed that humidity generally has a more significant impact on their viability than temperature, with humidity levels below 34% hindering egg survival (Jansen *et al*, 2021). Temperatures ranging from 5-25°C support egg viability, but temperatures exceeding 25°C and freezing conditions reduce egg survival. Actual environmental conditions, including types of parasites and ambient weather conditions, play a crucial role in egg survival. In natural grassland environments eggs can survive for up to one year (Jansen *et al*, 2021).

The prevalence of *Toxocara* spp contamination in fresh vegetables in our study at 1% is in concordance with the reported rate (3%) in the previous study conducted in Nakhon Si Thammarat Province (Punsawad *et al*, 2019). However, a broader range of the presence *Toxocara* spp in fresh vegetables was reported in other regions of the globe: in Ethiopia at 14-16% (Bekele *et al*, 2017; Bekele and Shumbej, 2019), Iran at 21% (3/14) (Taghipour *et al*, 2019), Iraq at 7% (Mirzaei *et al*, 2021), Jordan at 7% (Ismail, 2016), Lebanon at 1% (El Safadi *et al*, 2023), and Tunisia at 2% (M'rad *et al*, 2020).

Toxocara spp are the most frequently encountered parasitic organisms in dogs and cats globally (Phoosangwalthong *et al*, 2022). The presence of *Toxocara* spp in vegetables raises concerns about possible contamination with feces from dogs and cats, posing a risk of zoonotic transmission. This risk is particularly high for children, given their increased vulnerability to infection due to risky behavior and poor hygiene practices. The primary manifestations of toxocariasis are classified into two forms: visceral larva migrans (VLM) and ocular larva migrans, the former predominantly a disease of young children (Despommier, 2003).

We found lettuce to be the most helminth-contaminated vegetable (63%), in keeping with reports from other countries: Argentina at 59% (Falcone *et al*, 2023), Indonesia at 56% (Pratama *et al*, 2023), Jordan at 63% (Ismail, 2016), Lebanon at 23% (El Safadi *et al*, 2023), and Syria at 29% (Al Nahhas and Aboualchamat, 2020).

The high prevalence of helminth contamination in lettuce leaves compared to other vegetables may be attributed to their unique leaf structure. Unlike the smooth surfaces of many vegetables, lettuce possesses folds, crevices and a distinctly textured surface (Vizon *et al*, 2019; Al Nahhas and Aboualchamat, 2020), thereby facilitating the adhesion and trapping of parasite eggs and larvae. Although our result is at odds with earlier studies in Thailand, where celery in Nakhon Nayok Province (60%) and Nakhon Si Thammarat Province (63%) was the most contaminated vegetable (Punsawad *et al*, 2019; Krainara *et al*, 2023), it emphasizes the influence of vegetable structure on the presence of helminths. Yearlong beans exhibited the lowest helminth contamination at 15%, consistent with findings by Pratama *et al* (2023) in Indonesia. These findings might reflect the use of support structures in yearlong bean cultivation, thus elevating stems and pods above the ground, thereby reducing the likelihood of contact with soil helminth eggs or larvae. The impact of agricultural practices is also a crucial factor determining helminth prevalence in vegetables.

We observed a statistically significant different distribution of helminth species among the nine vegetable types in agreement with previous studies conducted in Bangkok on eight types of vegetables (Niamnuy *et al*, 2022) and in Nakhon Si Thammarat Province on ten types of fresh vegetables (Punsawad *et al*, 2019). This is not unexpected given the variations in the vegetable anatomical characteristics, agricultural practices, types and density of helminths in soils, and environmental factors affecting parasite growth and proliferation.

Future research endeavors should delve deeper into the abovementioned factors affecting helminth contamination of fresh vegetables in the various regions of the country. Such information would assist in developing strategies to minimize helminths' presence in fresh vegetables tailored for each agricultural region. Social aspects, such as farmers' hygienic practices, vegetable storage conditions, integrated pest management strategies, and consumers' preferences for the types of fresh vegetables, should also be taken into consideration. Simple methods of adequate washing of fresh vegetables before consumption should be widely disseminated, particularly in schools, which can provide a practical demonstration

of hygienic ways of preparing fresh vegetables as well as distributing educational material to take home.

In conclusion, our study revealed the prevalence of nine helminth parasites' contamination in fresh vegetables cultivated on farms in in Bang Khla District, Chachoengsao Province, Thailand. The potential transmission of human intestinal parasitic infections through the consumption of improperly prepared fresh vegetables grown in the study area is evident. Our findings emphasize the vital necessity for robust hygiene practices, including thorough washing with clean water and disinfection solutions, along with proper cooking techniques. Implementing these measures can ensure the consumption of vegetables devoid of helminth and other pathogenic contaminants.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

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