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PARASITIC CONTAMINATION OF RAW SALAD VEGETABLES IN LOCAL MARKETS OF DHAKA: HEALTH RISKS AND HYGIENE PRACTICES OF VENDORS

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Keywords: Bangladesh, hygiene practices, parasitic contamination, raw salad vegetables

Abstract

Salad vegetables are widely eaten raw in Bangladesh because of their high nutritional content and health benefits. However, contamination of salad vegetables with parasites poses a significant health risk. This study aimed to document the parasitic contamination of raw salad vegetables sold in local markets of Dhaka and the role of vendors' hygiene practices in transmitting parasitic pathogens. 29.6% of the salad vegetables were contaminated with 8 different parasites. *Ascaris* sp. (20.4%) > *Entamoeba* sp. (13.0%) > *Hymenolepis* sp. (11.1%) > *Cystoisospora* sp. (5.6%)> Hookworm (3.7%), *Trichuris trichiura* (3.7%) > *Taenia* sp. (2.8%). Leafy vegetables (coriander 66.7%, mint 44.4%, lettuce 38.9%) were more contaminated than non-leafy vegetables (carrot 11.1%, Tomato 11.1%, cucumber 5.6%). The vendors' hygiene practices: the habit of washing hands with soap and nail status, and infected vendors were proved to be the statistically significant contributing sources of observed parasitic contamination. The present study demonstrated the presence of infective stages of different pathogenic parasites on the surface of raw salad vegetables. It establishes a relationship between vendors' hygiene practices and parasite transmission. Eating them may spread vegetable-borne diseases in Dhaka unless adequately cleaned and disinfected prior to consumption.

Introduction

Salad vegetables are commonly referred to as a healthy nutritional diet due to their vast array of health benefits. Due to their abundance in amino acids, carbohydrates, fibers, vitamins, essential fatty acids, minerals, and other nutrients, salad vegetables have attracted interest from people worldwide[(1,2)]. They are essential and beneficial for preserving health and avoiding several disorders[(3)]. Vegetables are ideal for weight control due to their low-

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calorie and low-fat content. In addition to being nutrient-dense, salad vegetables are widely consumed due to their affordability and accessibility. Vegetables have the potential to nourish the continuously growing population worldwide[1]. Eating a balanced diet is crucial to avoid developing chronic and infectious diseases[4]. Epidemiologic research has shown that a balanced diet of vegetables, fruits, whole grains, and other bioactive substances helps prevent infectious and chronic illnesses. Eating fruits, vegetables, and grains has reduced the risk of non-communicable diseases[5].

Despite these benefits, the risk of parasitic contamination is a significant concern, particularly in raw salad vegetables. Raw salad vegetables are found to be contaminated with intestinal parasites, including protozoa and helminths. Numerous studies have documented the presence of intestinal protozoa, e.g., *Cryptosporidium* sp., *Giardia* sp., *Entamoeba* sp., *Cyclospora*, *Toxoplasma gondii*, *Cystoisospora belli*, *Balantidium coli*, *Blastocystis* sp., and intestinal helminths, e.g., *Ascaris lumbricoides*, *Strongyloides*, hookworm, *Trichuris trichiura*, *Enterobius vermicularis*, *Toxocara*, *Hymenolepis* sp., *Taenia* sp., *Echinococcus* from raw salad vegetables and leafy greens[6-9]. From the time they are grown until they are eaten, there is a risk that vegetables will be contaminated with parasitic, bacterial, or viral pathogens[10].

Pathogens have the potential to contaminate fresh vegetables "on-field" or during pre-harvesting in several different ways. Some of these ways include using untreated manure and compost, contaminated irrigation water, contaminated soil, insect transmission, or fecal contamination by people or animals[11-13]. Research shows that vegetables can get significantly contaminated with pathogens from raw or contaminated (or inadequately treated) manure [14,15]. It is possible for the pathogens that are present in manure to spread to vegetables either through the splashing of soil or manure particles from the soil onto vegetables as a result of rainfall, irrigation, or vectors or through direct contact between manure and the surfaces of vegetables[14]. Parasites occurring in soil are *Ascaris* sp., *Trichuris* sp., Hookworm, *Capillaria* sp., *Toxocara* sp., and Taeniid[16]. As a result, soil can act as a medium for the contamination of vegetables produced in polluted soil, either through the seeds, the roots, or the surfaces of the vegetables[17].

After the vegetables have been harvested, there is a risk of contamination due to contaminated wash water or cross-contamination from handlers affected by the pathogens[18]. According to the previous study's findings, unsanitary conditions prevailing during the transportation process might potentially contribute to the contamination of vegetables[9]. Raw vegetables are generally contaminated when harvested and transported and exhibited in containers that are not sufficiently cleaned and disinfected. This practice can lead to the contamination of salad vegetables[6]. Vegetable vendors' inadequate hygiene and preservation methods may increase contamination[19].

It has been known for a very long time that eating fruits and vegetables can provide nutritional and health advantages. Consequently, individuals are shifting from unhealthier to healthier eating patterns and increasing the proportion of fruits and vegetables[17]. Because fresh salad vegetables are frequently ingested raw, without peeling, or with minimal preparation, contamination with pathogens poses a considerable threat to human health[8, 20]. Salad vegetables, such as cucumber, carrot, tomato, lettuce, coriander, and mint, are widely eaten raw in our country because of their high nutritional content and numerous health benefits. Cucumbers, carrots, tomatoes, lettuce, and coriander are often consumed with street foods. In addition to this, coriander is often used in "Vorta" (mashed delicacy), contributing to its widespread popularity. Both juices and "Borhani" (a yogurt-based drink) are typically made with fresh mint.

The purpose of this study was to document the presence of protozoan cysts and helminth eggs on the surface of vegetables. This study aimed to provide insight into the level of parasitological contamination of salad vegetables sold in local markets of Dhaka, Bangladesh. Efforts to reduce contamination can be focused if the factors contributing to higher contamination levels are identified. We also surveyed vegetable vendors' hygiene practices to determine the probable sources of contamination. Insufficient information is available in Bangladesh on the parasitological quality of vegetables except for one conducted in the year 2000[21]. This study was conducted to add trustworthy information to existing knowledge and highlight the potential risks of an epidemic of vegetable-borne diseases related to consuming contaminated salad vegetables.

Material and Methods

Collection of samples

Vegetables were randomly collected from markets in Dhaka, including Jatrabari, Demra, and Wari, between March and December 2022. Six common salad vegetables were chosen: carrot, cucumber, tomato, coriander, mint, and lettuce, with 18 samples of each type. A total of 108 fresh salad vegetables were investigated. A Survey was designed to find the vendors' hygiene practices: the habit of washing hands with soap, nail status, the practice of washing vegetables, and the practice of storing vegetables. The vendor who frequently (at least once in three months) suffered from gastrointestinal diseases was also recorded. Vegetables were collected from 100 vendors.

Parasitological examination

Fresh samples were bought early in the morning and transported immediately to the laboratory in separate plastic bags. The parasitological examination was performed at "The Parasitology laboratory," Department of Zoology, University of Dhaka. Ten grams of the sample were weighed and immersed in 90 ml of saline water (0.9% NaCl) for two hours to release cysts and eggs attached to the surface of the vegetables. After two hours, the sample was removed gently, and the washing saline water was left overnight for sedimentation. The top water was slowly poured out, and the remaining washing saline water (about 10

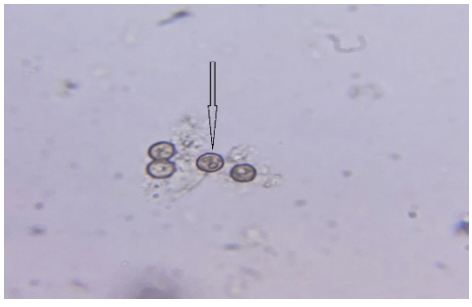
ml) was centrifuged for five minutes using a Low-speed Tabletop Centrifuge Machine (Model: HK-80-1, Manufacturer: TKA, Origin: China). The Relative Centrifugal Force (RCF) was 1006 g. The supernatant was discarded. A droplet of bottom water with silt was placed on a clean glass slide. A clean cover slip was delicately placed, avoiding bubbles. The prepared slide was examined under a Binocular Compound Light Microscope (Model: BB.1152-PLi; Manufacturer: Euromex, Origin: Netherlands). The slide was first inspected under 10x magnification for helminth eggs. Suspected eggs were then inspected under 40x magnification for identification. In the case of protozoan cysts, the slide was inspected under 40x magnification. Suspected cysts were then inspected under a 100x oil immersion lens for identification. Cysts and eggs were identified based on previous descriptions[16].

Statistical analysis

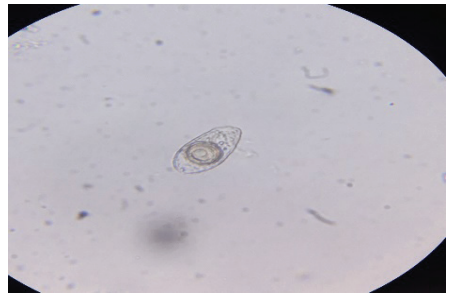
All statistical analyses were done using SPSS software version 25.0 for Windows (SPSS Inc., Chicago, IL, USA). $p < .05$ was considered statistically significant. Data visualization was performed using Microsoft Office Excel version LTSC (Microsoft Corporation, Redmond, WA, USA)

Results and Discussion

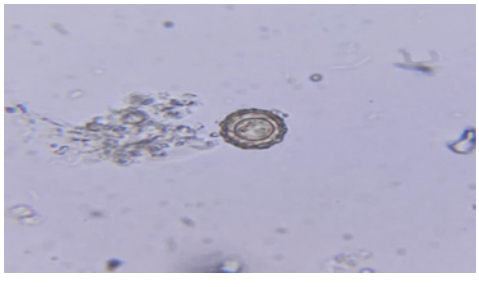
We recorded the presence of *Entamoeba* sp., *Cystoisospora* sp., *Hymenolepis* sp., *Taenia* sp., *Ascaris* sp., *Trichuris trichiura*, and Hookworm in raw salad vegetables (carrot, tomato, cucumber, coriander, mint, lettuce) sold in local markets of Dhaka city. Our study showed that 29.6% of the samples were contaminated with parasitic cysts, oocysts, and eggs. The possibility of parasitic illnesses spreading through food is becoming more widely recognized[22]. As a result, our study aimed to determine the degree of parasitic contamination of some commonly consumed raw salad vegetables in Dhaka. The finding is almost similar to other studies done in Syria[7], Egypt[22] and Iran[23]. The contamination rate in our current investigation was higher than the rate found in some earlier studies, including "ready-to-eat vegetables" in Iran[24], Nigeria[6], Egypt[25], and Turkey[26]. Some contributing variables could be behind such discrepancies, including the geographical location, the kind of samples, sample size, and techniques utilized to diagnose intestinal parasites. Variability in the types of vegetables evaluated in each study and the procedures for parasite recovery used in the various investigations were two of the most likely causes of the observed differences in the prevalence of parasitic organisms in vegetable samples[27].



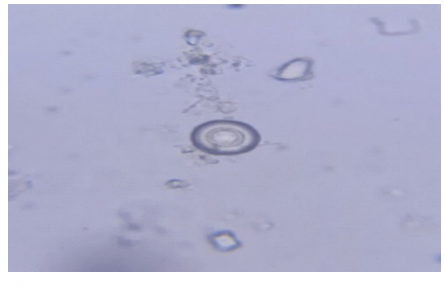
a) *Entamoeba* sp. (cyst)



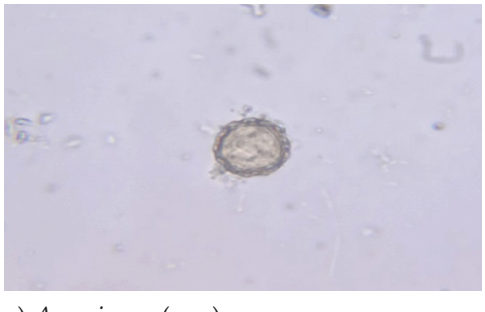
b) *Cystoisospora* sp. (oocyst)



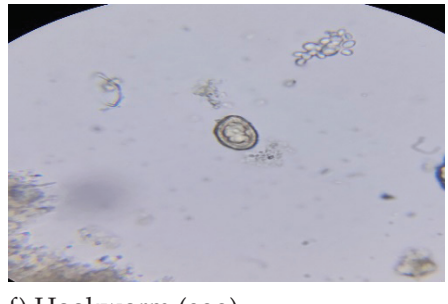
c) *Hymenolepis* sp. (egg)



d) *Taenia* sp. (egg)



e) *Ascaris* sp. (egg)



f) Hookworm (egg)

g) *Trichuris trichiura* (egg)

Fig. 1. Parasites detected and recorded from raw salad vegetable samples

(a) *Entamoeba* sp. (100X); (b) *Cystoisospora* sp. (40X); (c) *Hymenolepis* sp. (40X); (d) *Taenia* sp. (40X); (e) *Ascaris lumbricoides* (40X); (f) Hookworm (40X); (g) *Trichuris trichiura* (40X)

Ascaris sp. had the highest percentage of 20.4%, followed by *Entamoeba* sp. (12.9%) > *Hymenolepis* sp. (11.1%) > *Cystoisospora* sp. (5.6%) > Hookworm (3.7%) > *Trichuris* Sp. (3.7%)> *Taenia* sp. (2.8%). (Fig. 2).

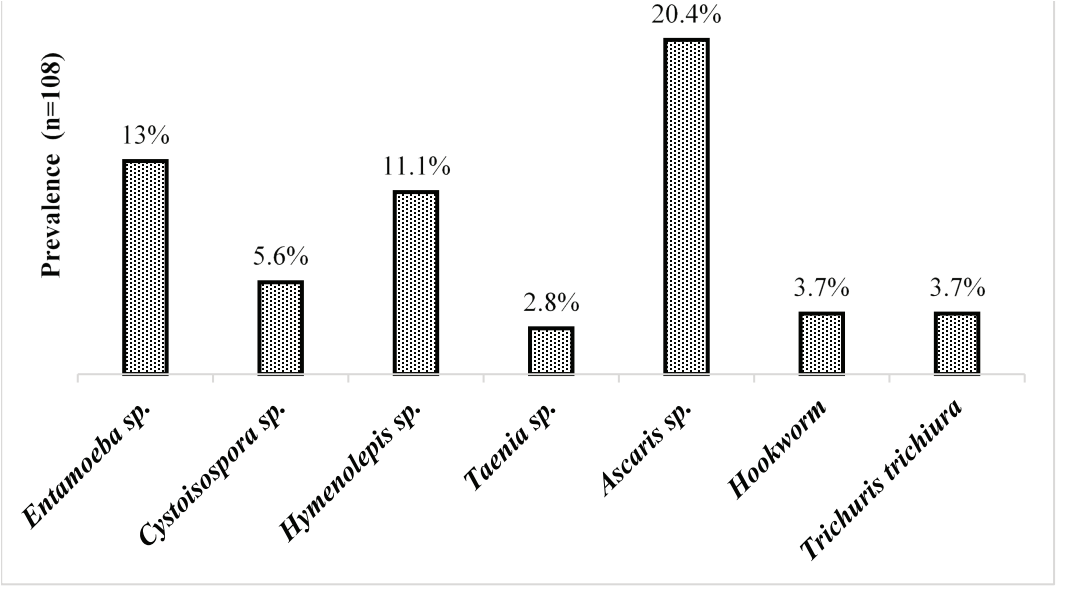


Fig. 2. Prevalence of parasites in salad vegetables

The percentage of *Ascaris* sp. (20.4%) in the current study aligns with the study from Egypt[22] but differs from some other studies showing either lower[6,7, 9] or higher prevalence [10]. The only study in Dhaka on raw vegetables showed a much higher prevalence of *Ascaris lumbricoides*[21] than ours. *Entamoeba* sp. had the percentage of 12.9%, which was inconsistent with some other studies, including in Birjand city of Iran[9], Khorramabad of Iran[28], and Egypt[25]. The overall percentage of *Hymenolepis* sp. (11.1%) was almost similar to a study in Iran[9]. The prevalence was higher compared to a study in Syria[7]. The prevalence of Hookworm (3.7%) in the present study differed from other studies, including in Nigeria[27] and India[29]. The prevalence of *Trichuris trichiura* (3.7%) was similar to studies in Iran[9] and in Nigeria[27]. The prevalence of *Taenia* sp. (2.8%) in this study was not identical to studies in Iran[9], Syria[7], Nigeria[27], Libya[10], and Turkey[26].

The most contaminated vegetable type was coriander (66.7%), followed by mint (44.4%) and lettuce (38.9%). Carrot (11.1%), Tomato (11.1%), and Cucumber (5.6%) were mildly contaminated (Fig. 3)

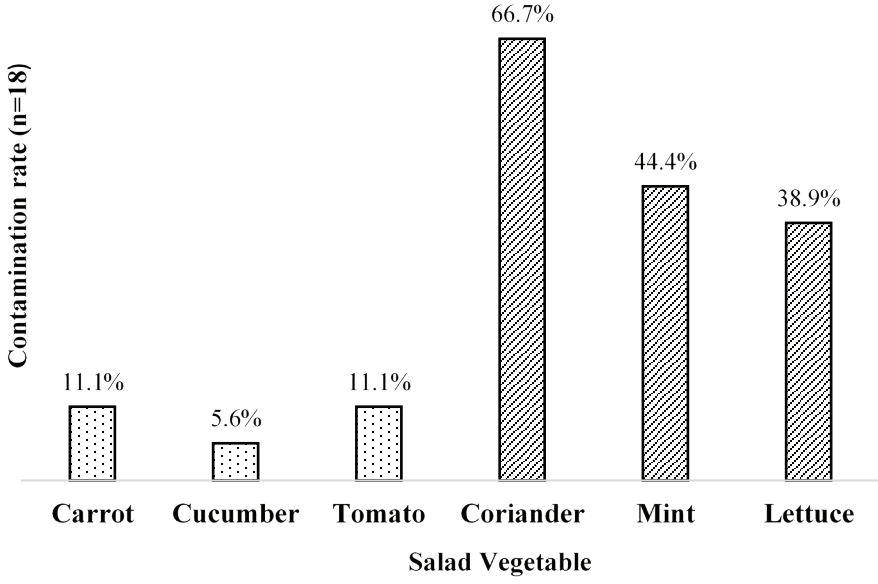


Fig. 3. Level of parasitic contamination of raw salad vegetables (Leafy: carrot, cucumber, tomato; non-leafy: coriander, mint, lettuce)

Among the leafy vegetables, coriander samples were contaminated with all types of parasites (Table 1). 55.6% of the coriander samples were contaminated with *Ascaris* sp.; 27.8% with *Entamoeba* sp.; 27.8% with *Hymenolepis* sp.; 16.7% with *Taenia* sp.; 11.1% with *Cystoisospora* sp. and 5.6% with *Trichuris* sp. and Hookworm. 22.2% of mint were contaminated with *Entamoeba* sp. and *Cystoisospora* sp.; 16.7% with *Ascaris* sp.; 11.1% with Hookworm; 5.6% with *Trichuris trichiura*, *Hymenolepis* sp. However, *Taenia* sp. was not recorded in mint samples (Table 1). 33.3% of the lettuce was positive for *Ascaris* sp.; 16.7% was positive for *Entamoeba* sp. and *Hymenolepis* sp.; 11.1% was positive for *Trichuris* sp.; 5.6% was positive for Hookworm. *Cystoisospora* sp. and *Taenia* sp. were not found in lettuce samples (Table 1).

The 5.6% of carrot samples were contaminated with *Entamoeba* sp., *Ascaris* sp., and *Hymenolepis* sp.; 5.6% of the cucumber samples were contaminated with *Entamoeba* sp. and *Ascaris* sp.; 5.6% of the tomato samples were contaminated with sp. and *Hymenolepis* sp. (Table 1). *Cystoisospora* sp., *Taenia* sp., Hookworm, and *Trichuris trichiura*, were absent in all of the samples of carrot, tomato, and cucumber (Table 1).

Our study showed that the most parasitic contamination was in leafy salad vegetables. Coriander was found to have the highest level of contamination (66.7%), followed by mint (44.4%) and lettuce (38.9%), respectively. This higher level of contamination of leafy vegetables might be attributable to the fact that the rough and wide surface area aided parasite cyst and ova attachment, resulting in contamination[24]. Additionally, leafy

vegetables had relatively short stems, which allowed for increased interaction with the soil and the irrigation water[26,30]. Smooth, leathery surfaces on vegetables like tomatoes and cucumbers slowed the incidence of parasite attachment[6,31].

Salad vegetables were collected from 100 vendors who sold vegetables in local markets of the study area. Among the vendors from whom the vegetables were purchased, it was found that 86.10% of the vendors stored water for a few days and used this water repeatedly to clean the vegetables. 10.2% of the vendors wash the vegetables with clean water, whereas 89.8% wash the vegetables with unclean water (Fig. 4). It was found that all (100%) of the vendors displayed vegetables in open, unclean baskets and stored vegetables at room temperature.

The 71.3% of the vendors had suffered from gastrointestinal diseases (at least once in the past three months), whereas 28.7% had not. Only 13.9% habitually washed their hands with soap, whereas 86.1% did not. 27.8% of the vendors had trimmed nails, but 72.2% had untrimmed nails (Fig. 4).

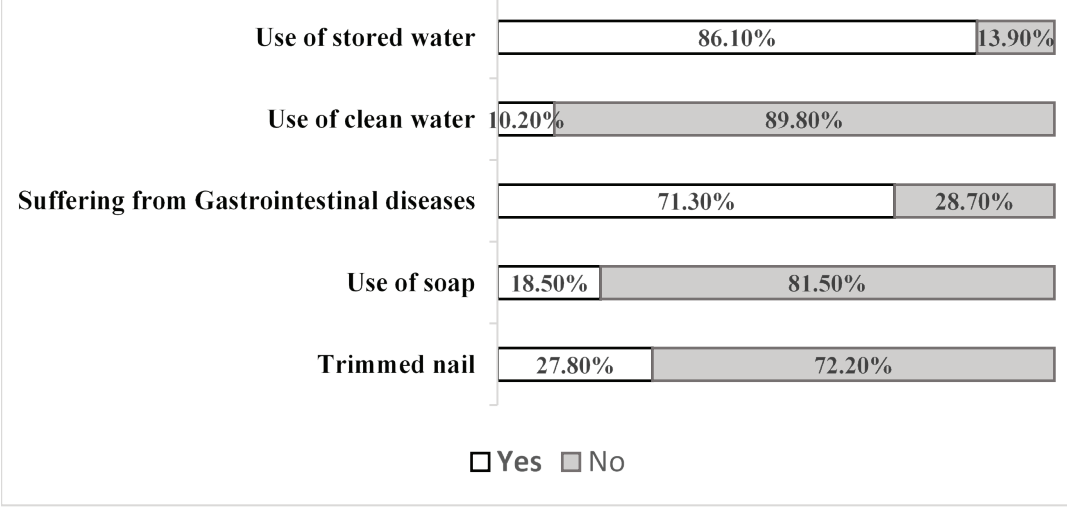


Fig. 4. Vendors' hygiene practices (n = 100)

We determined the statistical association between parasitic contamination and vendors' hygiene practices to establish vendors' hygiene practices as contributing factors to parasitic contamination. Parasitic contamination was related to cross-contamination from infected vendors (chi-square = 8.302, p = 0.004), from vendors who do not use soap (chi-square = 10.335, p <0.001), from vendors having untrimmed nails (chi-square = 13.776, p<0.0001).

Detecting infective stages of *Entamoeba* sp., *Taenia* sp., *Hymenolepis* sp., and *Ascaris* sp., in raw salad vegetables from Bangladesh is alarming. All of the identified parasites are well-known pathogens of humans. The study has provided evidence that establishes a significant relationship between parasitic contamination and vendors' hygiene practices.

Conclusion

Raw salad vegetables in Dhaka were contaminated with pathogenic parasitic cysts and eggs (*Entamoeba* sp., *Cystoisospora* sp., *Taenia* sp., *Hymenolepis* sp., *Ascaris* sp., *Trichuris trichiura*, and Hookworm), making the scenario very alarming. It was found that leafy vegetables, such as coriander, mint, and lettuce, contained significantly higher contamination levels. This study has demonstrated that vendors' hygiene practices could be a source of raw salad vegetable contamination. One possible conclusion that can be drawn is that consuming raw salad vegetables, especially leafy greens, maybe a means through which vegetable-borne parasitic diseases might be transmitted to humans in Dhaka. This poses a potential public health concern. To avoid potential outbreaks, vegetable vendors, along with consumers, must be conscious of hygiene practices and appropriate disinfection of salad vegetables before consumption.

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Table 1. Overall contamination rate of parasites in each type of raw salad vegetable

Vegetable	Number of samples(n)	*Entamoeba* sp.	*Cystoisospora* sp.	*Hymenolepis* sp.	*Taenia* sp.	*Ascaris* sp.	Hookworm	*Trichuris trichiura*
				Leafy Vegetable				
Coriander	18	27.8%	11.1%	27.8%	16.7%	55.6%	5.6%	5.6%
Mint	18	22.4%	22.4%	11.1%	ND	16.7%	11.1%	5.6%
Lettuce	18	16.7%	ND	16.7%	ND	33.3%	5.6%	11.1%
				Non-Leafy Vegetable				
Cucumber	18	5.6%	ND	ND	ND	5.6%	ND	ND
Carrot	18	5.6%	ND	5.6%	ND	5.6%	ND	ND
Tomato	18	ND	ND	5.6%	ND	5.6%	ND	ND

*ND = Not detected

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