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Prevalence of Parasitic Contamination of Locally Grown and Imported Fresh Leafy Vegetables Sold in an Open Market in Kuwait

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Abstract

Introduction: Various parasitic infections are transmitted via consumption of contaminated raw vegetables and fruits, and parasite species vary by geographic location, environmental conditions, and produce type. To our knowledge, there are no reports on the parasitic contamination of vegetables in Kuwait. This study aimed to determine the prevalence of parasites on various locally grown and imported fresh leafy vegetables in Kuwait. **Methods:** From February 2023 to February 2024, 180 fresh leafy vegetables were purchased from the central fruits and vegetable market and transported to the Research Core Facility, College of Medicine, Kuwait University. The vegetables were thoroughly washed, the rinsate centrifuged, and the pellet collected to prepare iodine-stained wet mounts and safranin-methylene blue smears. The specimens were examined for parasites by light microscopy and statistical analysis was performed using Microsoft Excel® Analysis ToolPak software. **Result:** Parasitic contamination was detected on 10 out of 180 (5.6%) vegetables, from which 10 parasite species were identified. The parasitic load was approximately 100 - 300 eggs/cysts/larvae per 300 grams of vegetable. The Jordanian lettuce had the highest parasitic contamination (3 out of 20, 15.0%) with 4 species (*i.e.*, *Hymenolepis diminuta*, *Entamoeba coli*, *Iodamoeba* spp., and *Anoplocephala* spp.), followed by Kuwaiti celery leaves (3 out of 20, 15.0%) with 3 species (*i.e.*, *Heterophyes heterophyes*, *Strongyloides stercoralis* larva, and *Sarcocystis* spp.), while 2 out of 20 (10.0%) Kuwaiti arugula bunches had *Cryptosporidium* spp. and *Giardia intestinalis* cyst. Only 1 out of 20 (5.0%) Kuwaiti parsley bunches was contaminated with *Eimeria* spp. oocyst, and 1 out of 20 (5.0%) Jordanian iceberg lettuce heads was contaminated with *Strongyloides* spp. egg containing larva. **Conclusion:** This is the first report on the prevalence of parasitic contamination of fresh leafy vegetables in Kuwait. These

findings highlight the importance of effective public health measures, including washing vegetables before consumption to reduce the risk of transmission to humans.

Keywords

Parasite, Prevalence, Contamination, Vegetables, Kuwait

1. Introduction

Foodborne diseases are caused by the consumption of food that is contaminated by infectious agents such as bacteria, fungi, viruses, and parasites, or non-infectious toxic chemicals such as pesticides, heavy metals, mycotoxins, and allergens. The foodborne parasitic infections of high global relevance based on the economic burden and the frequency and severity of infection include cryptosporidiosis, amoebiasis, ascariasis, giardiasis, and toxoplasmosis, while parasites of regional importance include *Taenia* spp., *Echinococcus* spp. *Fasciola* spp., and *Trichinella* spp. [1].

Various parasitic infections can be transmitted via consumption of contaminated raw vegetables and fruits. In recent years, *Cryptosporidium* emergence/reemergence has been associated with salad vegetables, probably due to the general rise in the per capita consumption of salad, the increase in popularity of prewashed ready-to-eat (RTE) salad vegetables and the rise in international trade of salad ingredients [2]. Several outbreaks of cryptosporidiosis have been associated with precut mixed salad leaves in European countries such as the UK, Spain, and France [3]-[5]. Giardiasis is also prevalent due to the use of contaminated water for crop irrigation and poor hygiene of food handlers, and *Giardia intestinalis* cysts have been reported on a variety of crops including lettuce, dill, bean and radish sprouts, and strawberries, from which the highest prevalence was reported in leafy vegetables [6]-[8]. In addition, studies from Europe and the Middle East have reported the prevalence of dog and cat roundworm (*Toxocara* spp.) eggs on raw vegetables [9] [10], while studies from Asian countries, India and Thailand reported higher prevalence of *Ascaris lumbricoides* and hookworm, and *Strongyloides stercoralis* and hookworm, respectively [11] [12]. A recent meta-analysis of thirteen studies from Ethiopia reported a pooled prevalence of 43.38% parasitic contamination in raw fruits and vegetables with at least one parasite, and the most prevalent parasite was *Strongyloides stercoralis*, followed by *Toxocara* spp., *G. lamblia* and *A. lumbricoides* [13]. Several studies from the regional Gulf countries, Egypt, Iran, Syria, Lebanon and Iraq have reported various degrees of parasite contamination rates of a variety of fresh vegetables and fruits [8] [14]-[17].

Kuwait is a small country (17,818 km^2) located in the northeastern part of the Arabian Peninsula which has an arid environment with long, hot, and dry summers and scarce rainfall. The land area is dominated by porous sandy soil which

is characterized by poor organic matter content and a high rate of water evaporation. The agricultural lands comprise only 8.4% of the total land area in Kuwait and the only source of freshwater is a few groundwater lenses [18]. Limited number of vegetables and fruits are grown in greenhouses using imported fertilizers and modern watering system. Consequently, various vegetables and fruits are imported from the regional developing Arab and Asian countries. Use of untreated human and animal waste as fertilizer and untreated water for irrigation is a common practice in these developing countries.

Kuwaiti cuisine is a fusion of Arabian, Mediterranean, Persian, and Indian dishes with a lot of fresh salads as side dishes. People typically eat out at restaurants on weekends. In addition, diet foods with fresh salads and vegetables are popular among the young population group.

Though the Ministry of Public Health Officials regularly inspects the market, to our knowledge, there is no previously reported data on the parasitic contamination of fresh vegetables sold in Kuwait. This study aimed to determine the prevalence of parasites in various locally grown and imported fresh leafy vegetables in Kuwait.

2. Material and Methods

2.1. Study Design and Sample Collection

A cross-sectional study was conducted on fresh vegetables collected from Al Forda Central Fruits and Vegetables Market in Sulaibiya Kuwait, the country's largest wholesale market for fresh produce.

From February 2023 to February 2024, a total of 180 locally grown and imported fresh leafy vegetable samples *i.e.*, equal numbers of lettuce (*Lactuca sativa*), iceberg lettuce (*Lactuca sativa* var. capitata), arugula (*Eruca vesicaria*), parsley (*Petroselinum crispum*), cilantro (*Coriandrum sativum*), and dill (*Anethum graveolens*) were randomly selected and purchased from the open market vendors. These vegetables were selected as they are commonly used as "ready to eat" salads without washing or cooking, thus posing a higher risk of infection. The vegetable type, country of origin, and date of collection were recorded, and the vegetables were transported in sealed sterile plastic bags to the Research Core Facility, College of Medicine, Kuwait University for processing.

2.2. Sample Processing and Screening

There are several published methods to analyze vegetable material for the presence of parasitic eggs. A series of methods were performed to standardize our sample processing method. We determined that the most effective method to detect parasites eggs on leafy vegetables was to peel off the vegetables into small pieces before rinsing rather than mincing or homogenizing them to small pieces as that led to many plant cells which interfered with the microscopy of the slides. An equal amount (300 grams) of each vegetable sample was peeled off, broken into small pieces, and thoroughly rinsed in a bowl containing 500 ml of filtered

water followed by vigorous shaking. Modified from Healy *et al.* [10], the rinsate was then collected and dispensed in 50 ml conical tubes, centrifuged at 2000 × g for 10 min, and the supernatant was discarded. The pellet was resuspended in 5 ml normal saline solution and 200 µl of Lugol's iodine solution was added to 2 ml of sample solution, from which 50 µl were transferred to a microscope slide and the whole slide was scanned for parasite eggs/cysts/larvae using the Zeiss Axio Imager M1 light microscope with AxioCam camera under 20x objective lens. The parasites were counted, examined for internal structures, and photographed under 40x objective lens.

Safranin-methylene blue staining was used as described previously [19] to facilitate the detection of coccidian parasites that are less than 20 µm in size. Briefly, 30 µl of sample in normal saline were smeared onto a microscope slide, left to air dry, and passed transiently over a flame. Then, the smear was fixed in 3% HCI in absolute methanol for 5 min and then washed with water. The smear was then stained with 1% aqueous safranin for 60 sec, heated thoroughly until steaming, and washed with water. Finally, the smear was counterstained with 1% methylene blue for 30 sec, washed with water, and blotted dry. The stained smear was then screened for parasites using 20x objective lens and the detected parasites which appeared deep pink to red against a blue background were photographed under 63x oil immersion objective lens. The images were then enhanced, and the parasite size was measured using netScope® Viewer software version 1.11.8543.20354. Parasite identification was confirmed by referring to the Centers for Disease Control and Prevention (CDC) DPDx image gallery and Google Lens image recognition technology.

2.3. Statistical Analysis

The parasitic load on the contaminated vegetables was reported as the number of eggs/cysts/larvae per vegetable unit (300 grams). Descriptive statistical analysis was performed, and the chi-square test was used to test the association between the frequency of parasitic contamination and the country of origin, and the association between the frequency of parasitic contamination and the vegetable type using Microsoft Excel® Analysis ToolPak software. A p-value < 0.05 was considered as significant.

3. Results and Discussion

From a total of 180 fresh leafy vegetables screened (120 locally grown + 60 imported), 10 samples were contaminated with an overall prevalence rate of 5.6% (**Table 1**). A total of 10 parasite species were detected in the contaminated vegetables and the parasitic load was approximately 100 - 300 eggs/cysts/larvae per 300 grams of vegetable. Apart from Jordanian lettuce which showed 2 parasite species (*Entamoeba coli* and *Anoplocephala* spp.) in the same lettuce head, all other contaminated samples had only 1 parasite species per sample.

By country of origin, 6 out of 120 (5.0%) locally grown vegetables in Kuwait tested positive for parasitic contamination in comparison with 4 out of 60 (6.7%)

contaminated vegetables from Jordan. However, the difference was not significant ($\chi^2 = 0.212$, $df = 1$, $p = 0.645$). The prevalence rate of parasitic contamination on locally grown fresh leafy vegetables in Kuwait (5.0%) was lower than that reported in other Middle Eastern countries, which reached 53.1% in Iran [14], but was comparable to the rates reported in other Arabian Gulf countries such as Saudi Arabia (7.8%) [20], Iraq (10.2%) [17] and UAE (15.1%) [21]. It is worth mentioning that climatic environment of other Arabian Gulf countries is very similar to that of Kuwait, and bulk of vegetables and fruits are imported to these countries from the regional developing Arab and Asian countries where untreated human and animal waste is commonly used as fertilizer and untreated water is used for irrigation. In addition, the majority of vendors at the open vegetable market in Kuwait were from developing Arab and Asian countries with sub-standard general personal hygienic and living status, which could also contribute to the contamination of vegetables.

Table 1. Parasitic contamination of locally grown and imported fresh leafy vegetables in Kuwait.

	Vegetable type	Country of origin	No. of samples	No. of contaminated vegetables	Parasite species
Imported	Lettuce	Jordan	20	3 (15%)	*Hymenolepis diminuta*, *Entamoeba coli*, *Iodamoeba* spp., *Anoplocephala* spp.
	Iceberg lettuce	Jordan	20	1 (5%)	*Strongyloides* spp.
	Iceberg lettuce	Oman	20	0	-
Locally grown	Celery	Kuwait	20	3 (15%)	*Heterophyes heterophyes*, *Strongyloides stercoralis*, *Sarcocystis* spp.
	Arugula	Kuwait	20	2 (10%)	*Giardia intestinalis*, *Cryptosporidium* spp.
	Parsley	Kuwait	20	1 (5%)	*Eimeria* spp.
	Lettuce	Kuwait	20	0	-
	Cilantro	Kuwait	20	0	-
	Dill	Kuwait	20	0	-
Total	-	-	180	10 (5.6%)	-

Distribution of Parasite Contamination on Vegetables

By vegetable type, Jordanian lettuce showed both the highest parasitic prevalence rate (3 out of 20, 15%) and the highest parasitic diversity with 4 species detected (*i.e.*, *Hymenolepis diminuta*, *Entamoeba coli*, *Iodamoeba* spp., and *Anoplocephala* spp.) (**Figures 1(a)-(d)**).

This finding is comparable to an earlier study from Jordan which reported a high parasitic contamination rate of 29% in 133 salad vegetables screened, from which the highest contamination rate was found on lettuce samples (63%), which was significantly higher in comparison with tomato, parsley and cucumber ($p = 0.006$, Odds ratio = 4.6; $p = 0.001$, Odds ratio = 5.5 and $p < 0.0001$, Odds ratio = 46.6, respectively [22]. Interestingly, several studies from regional countries also reported that lettuce had the highest parasitic contamination rate in comparison with other vegetables, including Iran (91.1%; [14], Egypt (45.5%; [8], Syria (29.5%; [15], Lebanon (23.3%; [16], and Iraq (15.0%; [17]. While only 1 Jordanian iceberg

lettuce head out of 20 (5%) was contaminated with *Strongyloides* spp. egg containing larva (Figure 1(e)), no parasitic contamination was found on any iceberg lettuce samples imported from Oman. A study from India reported a parasitic contamination rate of 19.72% on fruits and vegetables collected from local markets in Tiruchirapalli, Tamilnadu and tomatoes, carrot and beetroot were the most frequently contaminated fruits and vegetables with diverse types of parasites [11].

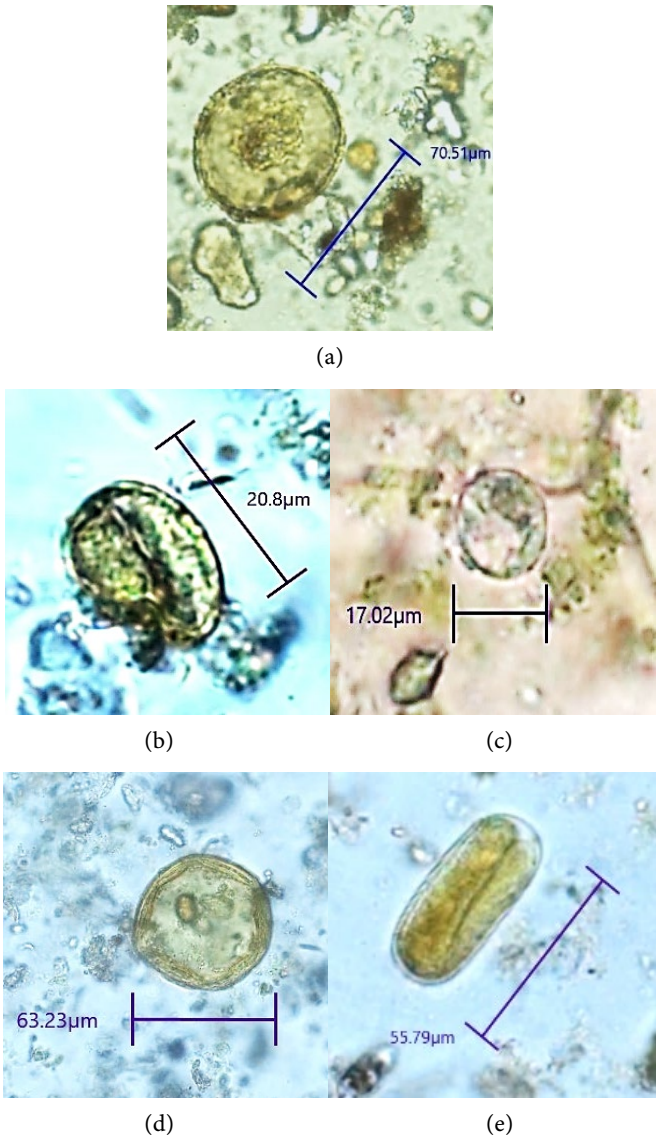

Figure 1. Parasites detected on imported fresh leafy vegetables by iodine-stained wet mount. Jordanian lettuce: (a) *Hymenolepis diminuta* egg, (b) *Entamoeba coli* mature cyst, (c) *Iodamoeba* spp. cyst, (d) *Anoplocephala* spp. egg. Jordanian iceberg lettuce: (e) *Strongyloides* spp. egg containing larva.

Kuwaiti celery leaves had the same prevalence of parasitic contamination as Jordanian lettuce (3 out of 20, 15%), with 3 species detected (*i.e.*, *Heterophyes heterophyes*, *Strongyloides stercoralis* larva, and *Sarcocystis* spp.) (Figures 2(a)-(c)).

Although Kuwait is considered non-endemic for strongyloidiasis, several cases

have been reported previously; two studies reported 6 cases of fatal strongyloidiasis hyperinfection following kidney transplantation from South Asian donors in conjunction with immunosuppressive therapy [23] [24], while the third study reported 4 cases (2 Kuwaiti, 1 Iraqi, and 1 Bangladeshi) of disseminated strongyloidiasis following the prescription of prednisolone and other steroids for pre-existing health conditions [25]. Kuwaiti farmers commonly employ workers from *Strongyloides*-endemic South Asian countries such as India and Bangladesh. Hence, detecting *Strongyloides* spp. on Kuwaiti celery may be attributed to open-field defecation and/or expectoration by infected workers, or may indicate the use of untreated soil or animal manure fertilizer contaminated with *Strongyloides* spp. eggs.

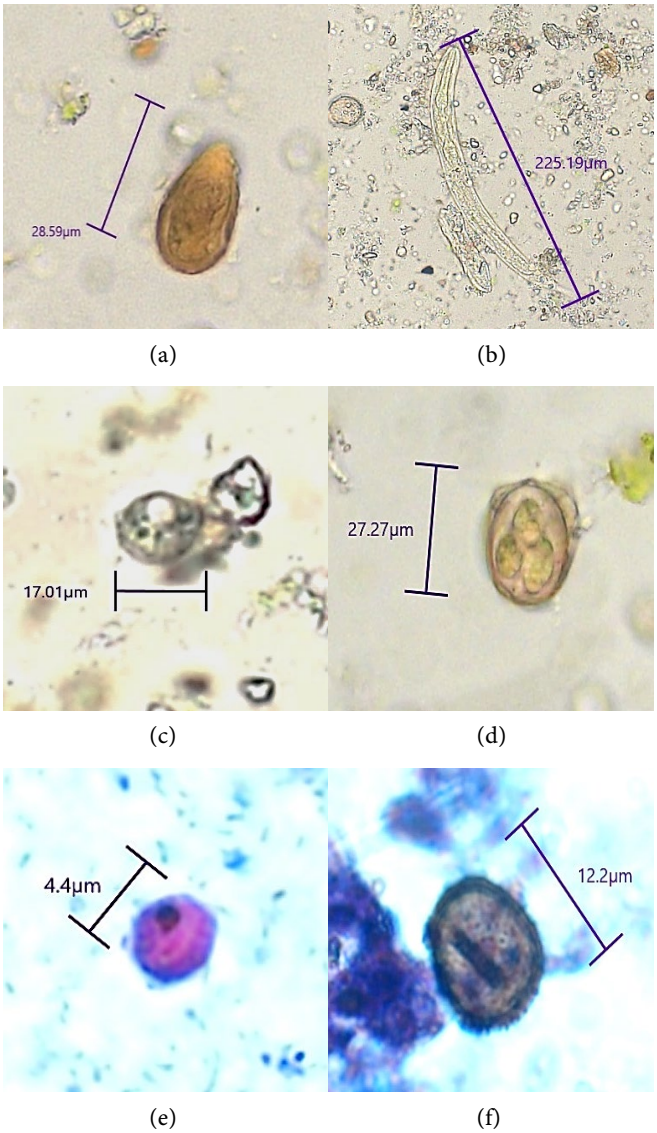

Figure 2. Parasites detected on locally grown fresh leafy vegetables by iodine-stained wet mount. Kuwaiti celery: (a) *Heterophyes heterophyes* egg, (b) *Strongyloides stercoralis* rhabditiform larva, (c) *Sarcocystis* spp. sporocyst. Kuwaiti parsley: (d) *Eimeria* spp. oocyst. Parasites detected on Kuwaiti arugula by safranin-methylene blue stain: (e) *Cryptosporidium* spp. oocyst, (f) *Giardia intestinalis* cyst.

To our knowledge, no human cases of sarcocystosis or heterophyiasis have been reported previously in Kuwait. However, an earlier study screened 240 stray cats for enteric parasites and detected *Sarcocystis felis* oocysts in 1 out of 240 (0.4%) fecal specimens [26]. Another study from Kuwait examined 240 stray cats for trematode infections and reported an overall prevalence rate of 24.6%, from which *Heterophyes heterophyes* was the most frequently reported (15.8%) trematode among 13 other species identified [27]. Thus, the detection of both *Sarcocystis* spp. and *Heterophyes heterophyes* on Kuwaiti celery samples in this study warrants an investigation into the prevalence of these parasites in stray cats located inside and around Kuwaiti farms.

This study detected parasites on 2 out of 20 (10%) Kuwaiti arugula bunches; 1 had *Cryptosporidium* spp. while the other had *Giardia intestinalis* cyst, and both were seen in safranin-methylene blue smears (**Figure 2(d)**, **Figure 2(e)**). In Kuwait, *Cryptosporidium* spp. and *Giardia duodenalis* infections have been reported previously among Kuwaiti children suffering from diarrheal illness with prevalence rates of 3.4% and 0.2%, respectively [28], while a more recent retrospective study reported *Giardia duodenalis* infection in 0.8% of hospital patients of all ages [29]. A veterinary study in Kuwait reported a high prevalence of *Cryptosporidium* spp. infection among pre-weaned calves in dairy cattle farms with a rate of 33.1% [30], a markedly higher rate than that in humans. Accordingly, it is more likely that the *Cryptosporidium* spp. detected on Kuwaiti arugula were transmitted by infected animals to the soil which was used to cultivate these vegetables. It is important to note that the safranin-methylene blue stain was of limited use in this study since the safranin dye commonly stained non-parasitic structures such as pollen grains and other artifacts of comparable size with coccidian parasites. Therefore, we do not recommend the use of safranin-methylene blue staining on samples of high biological diversity collected from outdoor environments (e.g., soil, water, vegetables and fruits), as these samples often harbor pollen grains, fungal spores and conidia, dinoflagellates, and microalgae.

Only 1 out of 20 (5%) Kuwaiti parsley bunches were contaminated with *Eimeria* spp. oocyst (**Figure 2(d)**). While there are no reports on human cases of *Eimeria* spp. infection in Kuwait, a previous study screened fecal specimens of farm animals for parasites and reported *Eimeria* spp. as the most common parasite in cattle, sheep, and goats [31]. Therefore, the parasitic contamination of locally grown arugula and parsley with *Cryptosporidium* spp. and *Eimeria* spp., respectively rather than transmission from an infected human. It was noted that some farmers used animal manure as organic fertilizer for vegetables, especially those who could not afford or had the facility of greenhouse plantation or contamination may have been transmitted by the unhygienic practices of the workers at the forms or vendors at the market, who had come from the regional developing Arab and Asian countries. All the locally grown lettuce, cilantro, and dill samples tested negative for parasitic contamination, which may be attributed to a variation in parasitic burden between individual farms. Accordingly, we expect that future surveillance

studies which incorporate a larger sample size of fresh vegetables including fruits collected from different farms in Kuwait will reveal a higher prevalence of parasitic contamination. According to a recent report from the Ministry of Public Health >46% of the population, especially older children and young adults are obese in Kuwait recommending public health awareness on weight control strategies including healthy diet containing fresh salads and fruits. Detecting parasitic contamination on locally grown vegetables in Kuwait paired with an ever-rising trend in RTE salad marketing and consumption necessitates routine surveillance of parasitic contamination on RTE raw foods. This study highlights the importance of effective public health measures, including personal hygiene and vegetable washing, to reduce the risk of transmission of infection by consumption of vegetables.

4. Conclusion and Recommendations

To our knowledge, this is the first report on the prevalence of parasitic contamination of locally grown and imported fresh leafy vegetables sold in the open market in Kuwait. Despite the stressful environmental conditions for parasite survival and growth, 10 parasite species were detected in the local and imported fresh vegetables, which could pose a risk of transmission of foodborne parasites to the consumers. These findings highlight the importance of effective public health measures including washing vegetables before consumption to reduce the risk of transmission to humans. We recommend that: 1) the peeling off and breaking vegetables into small pieces was found to be a better technique than the mincing of vegetables into small pieces with a knife for the detection of parasite eggs/larvae as that led to many plant cells interfering with the microscopy. 2) the safranin-methylene blue stain was of limited use in this study as the safranin dye commonly stained non-parasitic structures such as pollen grains and other artifacts of comparable size with coccidian parasites. 3) a larger-scale investigation of parasite contamination of fresh vegetables including fresh fruits using additional molecular techniques should be undertaken to inform food safety standards and safeguard consumer health. 4, highlight the importance of effective public health measures, including personal hygiene and vegetable washing, to reduce the risk of infection transmission to humans by consumption of unwashed vegetables.

Authors Contribution

MA and JI conceived the study and designed the experiments. MA collected the samples and conducted the experiments and performed then analysis. MA and JI drafted and reviewed the manuscript before submission.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

RTE: ready-to-eat

spp.: species

CDC: Center for Disease Control

DPDx: Division of Parasitic Diseases Diagnosis