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Original Article

Investigating the Effect of Four Different Washing and Disinfection Methods on Parasitic Contamination of Vegetables

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Abstract

Background: Raw vegetables are an essential part of a healthy diet, but when consumed without proper washing, they can serve as carriers of parasitic infection. An experimental study was conducted in Bushehr to investigate the parasitic contamination of raw vegetables and the effect of four different washing methods on their contamination.

Methods: A total of 299 fresh vegetable samples from 10 types of leafy and salad vegetables, including parsley (30), leeks (30), mint (30), basil (30), cress (30), radishes (29), purslane (30), lettuce (30), green bell peppers (30), and edible mushrooms (30), were purchased from 23 vegetable and fruit wholesale or retail stores located in different geographical areas of Bushehr city during three different seasons (2017–2018). The samples were divided into two groups: one was left unwashed (method 1), and the other was washed using a traditional washing method (method 2). Microscopic examination for the presence of parasites was performed on a direct wet mount. Then, positive samples were further subjected to a 3-Step washing method (water, detergent, water—method 3) and a 4-Step standard washing method (water, detergent, disinfectant, water—method 4), respectively. Parasitic contamination was investigated in the samples washed using all methods.

Results: Of the 299 examined samples, parasites were observed in 8.4% of the unwashed samples and 3% of the traditional washing method samples, and not in samples after washing with methods 3 and 4. A significant relationship was found between parasitic contamination and washing methods. All types of vegetables tested had at least one parasitic contamination. The highest rate of parasitic contamination was found in cress (20%), and the lowest contamination was found in leeks (3.3%). The highest rates of seasonal parasitic contamination were found in autumn (12.8%).

Conclusion: Parasitological contamination in unwashed and traditionally washed raw vegetables may pose health risks to consumers. Public education on proper vegetable washing methods is recommended to raise community awareness about the health risks associated with consuming unwashed or inadequately washed contaminated raw vegetables.

Keywords: Bushehr, Iran, Parasitic contamination, Vegetables, Protozoa, Nematode

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Introduction

Fresh vegetables play an important part in a healthy diet and are recognized as a vital source of nutrients necessary for human health (1-3). They also improve food quality due to their rich nutrient content, including water, dietary fiber, vitamin C, carotene, iron, and various vitamins such as thiamine (vitamin B12), niacin, and riboflavin. Their regular and continuous consumption has been shown to reduce the risk of cardiovascular disease, heart attack, and cancers (2). However, raw and improperly washed fruits and vegetables, often eaten without peeling, act as carriers for the transmission of various microbial flora, including intestinal bacteria and pathogenic parasites (1, 2, 4). The parasites most commonly linked to vegetable-borne and fruit-borne infection outbreaks include *Giardia lamblia* and *Ascaris lumbricoides* (5).

According to the FAO/WHO (Food and Agriculture Organization/World Health Organization) risk-ranking program (2012), 11 important parasites are transmitted through the consumption of fresh products (6). The FAO also reports serious concerns about the risk of contaminant transmission via vegetables (7). More than 40

million people are affected globally, and more than 10% of the world's population are at risk of contamination. One of the most important features of these organisms is their ability to produce cysts or larvae, which help their survival (5). At least 20 million hectares of agricultural land in 50 countries are irrigated with raw or poorly treated wastewater, increasing the risk of infectious diseases in highly contaminated areas (8, 9).

Statistics show a rise in the number of foodborne illnesses linked to fresh vegetable consumption. Several factors can cause food contamination, which can occur at different stages: on the farm, during different stages of production (planting), harvesting, handling, storage, distribution, selling, and even at the point of consumption (whether at home or in restaurants) (2, 4, 10).

Using disinfectants is one of the most important ways to prevent diseases (4). Studies have investigated the effectiveness of disinfections on reducing microbial contamination of vegetables and fruits (4, 10, 11). To prevent the transmission of parasites and related diseases from vegetables, substances such as perchlorine powder, commercially available disinfectants, detergents with benzalkonium chloride, and household detergents are commonly used for sanitizing vegetables in domestic and public settings (11). The Iranian Ministry of Health and Medical Education has introduced a four-step vegetable washing protocol. Step 1: Clean the vegetables in order to remove excess soil and mud and rinse them with clean water; Step 2: Soak the vegetables in water mixed with 3 to 5 drops of dishwashing liquid per 5 liters (for de-parasitizing); Step 3: Disinfect using 70% perchlorine or other available commercial disinfectants; Step 4: Rinse again thoroughly with clean water (12).

Numerous studies worldwide, including studies from Turkey (5), India (9), Nigeria (8), Egypt (2, 13), Sudan (3), and Italy (14), have shown that leafy or salad vegetables and fruits can be important sources of protozoan oocysts and cysts or parasitic worm eggs and larvae. A systematic review by Abdi et al identified 43 studies on parasitic contamination of raw vegetables in Iran (15). Only one study in this review was conducted in Bushehr (16), and it investigated a few raw leafy vegetables.

Approximately 75% of the agricultural lands in Bushehr Province use traditional irrigation, 25% use pressurized systems, and most irrigation depends on groundwater (from around 13,000 wells)(17). Jafari et al report that semi-deep wells are commonly used in Dashti County to access groundwater, primarily for cultivating garden crops (e.g., dates), cereals, vegetables, industrial crops, forage, and oilseeds (18). However, in some regions of Bushehr Province, irrigation by river water contaminated with municipal and industrial wastewater has been reported in addition to groundwater farmland irrigation (19, 20).

Due to Bushehr's hot and humid climate, some fruits and vegetables consumed locally are sourced from neighboring provinces like Khuzestan, Fars, and Hormozgan. These provinces, along with Azerbaijan, Kerman, Isfahan, Khorasan Razavi, and Hamedan, are among Iran's major vegetable-producing regions (21). However, several studies have reported the presence of parasitic infections in vegetables from neighboring provinces such as Fars (22) and Khuzestan (23). Notably, Bushehr ranks second in the country for the highest rate of vegetable consumption (24). Given that raw vegetables consumed by the people of Bushehr are supplied from various local and external sources depending on the season and considering that both geographical location and the time of study can influence the prevalence of parasitic contamination and its related factors for health interventions, this study aimed to investigate the parasitic contamination in raw vegetables sold in Bushehr and to evaluate the effectiveness of four different washing methods.

Methods and Materials

Sample Collection

This research is an experimental study conducted in Bushehr city during three different seasons (autumn, spring, and winter) in 2017–2018. A total of 299 fresh vegetable samples from seven types of leafy vegetables, including parsley (30), leeks (30), mint (30), basil (30), cress (30), radishes (29), purslane (30), and three types of salad vegetables, including lettuce (30), green bell peppers (30), and edible mushrooms (30), were purchased from 23 vegetable and fruit wholesale or retail stores located in different geographical areas of Bushehr. These stores mainly sold these types of products, and this is one of the main reasons for selecting them. Samples were randomly obtained from these shops.

Sample Preparation

Vegetable samples (1 to 2 kg of each type) were purchased and immediately transferred to the laboratory. To apply the four different washing methods, the edible parts of each sample were separated, and any decayed or non-edible parts were discarded. The remaining portion was then divided into two equal portions: one was left unwashed (method 1), and the other was washed using a traditional washing method (washing with clean water only, method 2).

If the unwashed (no-rinse) samples or samples processed by the traditional washing method tested positive for parasitic contamination, methods 3 and 4, which used detergent, disinfectant, and rinsing with clean water, were also performed, as described in Table 1.

Following the Ministry of Health's instructions (12), a commercially available benzalkonium chloride-based disinfectant, commonly used by the public, was used instead of perchlorine (12, 25, 26). In the de-parasitizing step (method 3), the vegetables were soaked in a solution of 3 to 5 drops of regular household dishwashing liquid

Table 1. The four washing methods and the steps of the Iranian Ministry of Health instructions

Method	Steps / Washing methods	without water	water	detergent*	Disinfectant**	water	Descriptions
1	Unwashed						-
2	Traditionally washing						Equal to step 1 of the Iranian Ministry of Health instructions
3	3-Step washing method						Equal to steps 1, 2, and 4 of the Iranian Ministry of Health instructions: Washing with water + detergent + water
4	4-Step washing method						The Iranian Ministry of Health standard washing method: Washing with water + detergent + disinfectant + water

*The type of detergent used was a common household dishwashing liquid.
** The type of disinfectant used was a high consumption commercial benzalkonium chloride-based solution.

per 5 liters of clean water for 5 minutes. In the disinfection step (method 4), the benzalkonium chloride-based disinfectant was added to 5 liters of clean water, and the vegetables were soaked in it for 5 minutes, followed by a final rinse with clean water.

Parasite Detection

Each of the initial weighted samples was placed in 9% saline solution and left for 45–60 minutes. After discarding the vegetables, the residual solution was allowed to rest for 24 hours, the supernatants were discarded, and the remaining part was carefully decanted into 15 mL Falcon tubes and centrifuged at 2000 rpm for 15 minutes. The supernatant was discarded again, and finally, the sediment was used to prepare direct wet mounts. Microscopic examination for determining the presence of parasites was performed on a direct wet mount. A drop of the mixture was transferred to the center of a clean glass slide and covered gently with a clean cover slip. The preparation was examined under a light microscope to detect parasites (eggs, cysts, and larvae) using × 10 and × 40 objective lenses (10).

Statistical Analysis

All data were entered and analyzed using SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Categorical variables (e.g., presence of parasitic contamination, type of washing method, vegetable type, and season) were summarized as frequencies and percentages. The chi-square test was applied to assess the association between parasitic contamination and other categorical variables, such as vegetable type and season of sampling. Fisher's exact test was used as an alternative to the chi-square test when dealing with small sample sizes or when the expected cell frequencies were low (27). *p*-values less than 0.05 were considered statistically significant, indicating a meaningful association between variables.

Results

Of the 299 examined samples, parasitic contaminations were observed in 8.4% (25/299) of the unwashed samples and 3.0% (9/299) of the traditional washing method samples. No parasite (0/34) was observed in the samples washed using the 3-Step washing method (water + detergent + water) and the 4-Step washing method (standard washing method) (Table 2). Furthermore, 56.0% of the contaminations in unwashed vegetables were related to nematodes (14/25) and 28.0% to protozoan trophozoite (8/25). In the traditional washing stage, 55.0% (5/9of the infections were related to nematodes and 45.0% (4/9) to protozoan cysts (Table 3).

The highest rate of parasitic contamination was in cress, 20.0% (6/30), followed by purslane and green bell pepper, 10.0% (3/30), and the lowest contamination rate was in the leek samples, with 3.3% (1/30) (Table 3). The highest rates of seasonal parasitic contamination were found in autumn 2018, spring 2018, winter 2018, and winter 2017, equal to 12.8%, 10.8%, 6.3%, and 3.3%, respectively (Table 4). However, there was no significant correlation between the type of vegetable and parasitic contamination ($P = 0.622$) or the sampling season with parasitic contamination ($P = 0.22$). The comparative analysis of vegetable washing methods revealed statistically significant differences in parasitic contamination levels among the tested protocols. Importantly, chi-square analysis revealed that unwashed vegetables had a significantly higher rate of contamination compared to those washed using the traditional method (washing with water alone) ($P = 0.008$). However, Fisher's exact test indicated that both the 3-step (water + detergent + water) and 4-step (standard washing method) washing methods were significantly more effective than the unwashed method ($P = 0.004$ for both comparisons). Moreover, based on Fisher's exact test, no statistically significant difference was observed between the traditional washing method and either the 3-step or 4-step methods ($P = 0.640$ for both comparisons). While the traditional method may reduce contamination, it does not achieve the same level of effectiveness as the structured multi-step protocols. Furthermore, no contamination was detected in either the 3-step or 4-step methods, indicating that both methods are equally effective in parasite removal.

The microscopic image of some of the larvae and trophozoites observed in the green specimens is shown in Figures 1 and 2.

Discussion

In the present study, the parasitic infections were identified in 8.4% of the unwashed samples, which is in line with findings from the study conducted in Asadabad city, west of Iran (28); however, other studies conducted in Iran (29), Ethiopia (30), and Sudan (3) have reported different results, and the highest contamination rate was observed in Shahroud, Semnan, Iran, equal to 38% (29). The results of a systematic review and meta-analysis study, which was done on 43 vegetable parasitic infection studies in Iran, reported an average contamination of 37% (15). Therefore, the result of our study is much lower than the average national rate. The reported parasitic infection rates in vegetables in Thailand, Egypt, Ethiopia, Sudan, and Nigeria are 35.1%, 31.7%, 25.1%, 10.6%, and 5.9%, respectively (2, 29, 31-33).

The comparison of the results of this study with the results of a parasitic prevalence survey in 1999 (15) on 17 crude edible vegetables (136 samples, prevalence 13.0%) in Bushehr shows a decrease in the rate of parasitic contamination in raw leaf vegetables in the last 20 years. In the recent decades in Iran, the proper implementation and monitoring program has been legislated and implemented to control the farms and agricultural lands under the supervision of Iran's Ministry of Health and Ministry of Agriculture to prevent irrigation with raw human and animal wastewater or fertilizers (34); this may be a one reason why the prevalence rate of parasitic contamination in raw vegetables in Bushehr has decreased. However, some studies have shown that vegetables are possibly still contaminated through contact with pets, other people, rodents, insects, and improperly treated wastewater (35).

The highest infection rate in the examined vegetables was observed in cress (20%), and the lowest rate was in leeks (3.3%). Another study similarly showed that cress and parsley were the most parasitically contaminated vegetables in Golestan, Iran (36). Probably the higher contamination rate of cress compared to other vegetables in our study is because its shorter stems help it carry more soil, which can potentially contain parasites that enter

Table 2. Parasitic contamination of vegetables based on different washing methods

Washing methods	Category	*n* (%)
Unwashed	Contaminated	25 (8.4)
	Non- contaminated	274 (91.6)
	Total	299 (100.0)
Traditionally washing	Contaminated	9 (3.0)
	Non- contaminated	290 (97.0)
	Total	299 (100.0)
3-step washing method	Contaminated	0 (0.0)
	Non- contaminated	34(100.0)
	Total	34(100.0)
4-step Ministry of Health instructions	Contaminated	0 (0.0)
	Non- contaminated	34 (100.0)
	Total	34 (100.0)

Table 3. Distribution of parasites between the washed and unwashed vegetables

Washing methods	Type of parasites	n (%)
Unwashed	Nematode	14 (56.0)
	Protozoa cyst	2 (8.0)
	Protozoa trophozoite	7 (28.0)
	Protozoa cyst & trophozoite	1 (4.0)
	Protozoa trophozoite & nematode	1 (4.0)
	Total	25 (100.0)
Traditionally washed	Nematode	5 (55.0)
	Protozoa trophozoite	4 (45.0)
	Total	9 (100.0)

Table 4. Association between parasitic contamination in unwashed vegetables, the type of vegetables, and seasons

Variable	Category	Number of examined samples	Rate of parasitic contamination *n* (%)	*χ2*	*P* value
Type of vegetables	Parsley	30	2 (6.7)	7.147	0.622
	Leek	30	1 (3.3)		
	Mint	30	2 (6.7)		
	Basil	30	2 (6.7)		
	Cress	30	6 (20.0)		
	Radish	29	2 (6.9)		
	Purslane	30	3 (10.0)		
	Mushroom	30	2 (6.7)		
	Lettuce	30	2 (6.7)		
	Green bell pepper	30	3 (10.0)		
Seasons of sampling	Winter 2017	60	2 (3.3)	4.414	0.22
	Spring 2018	120	13 (10.8)		
	Autumn 2018	39	5 (12.8)		
	Winter 2018	80	5 (6.3)		

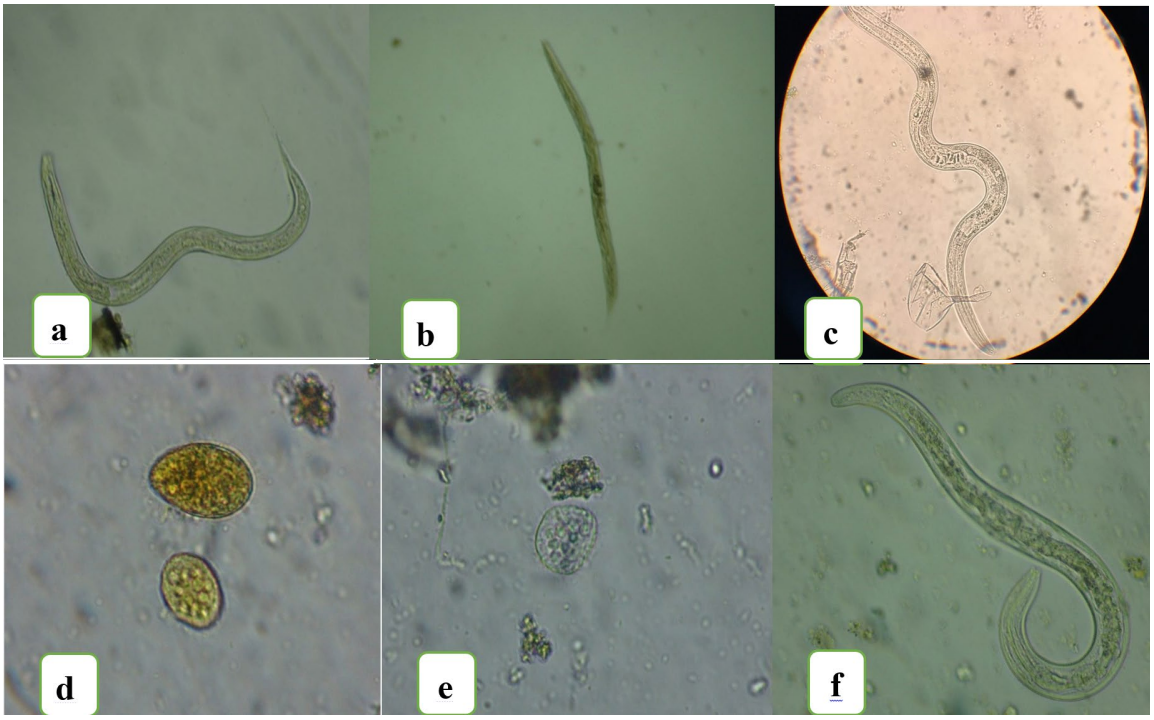

Figure 1. Microscopic slides of parasitic contamination isolated in leafy vegetables. **a** Cress nematode; **b** Parsley nematode; **c** Mint nematode; **d** Radish cyst; **e** Parsley cyst; **f** Purslane nematode; (original picture, 400X)

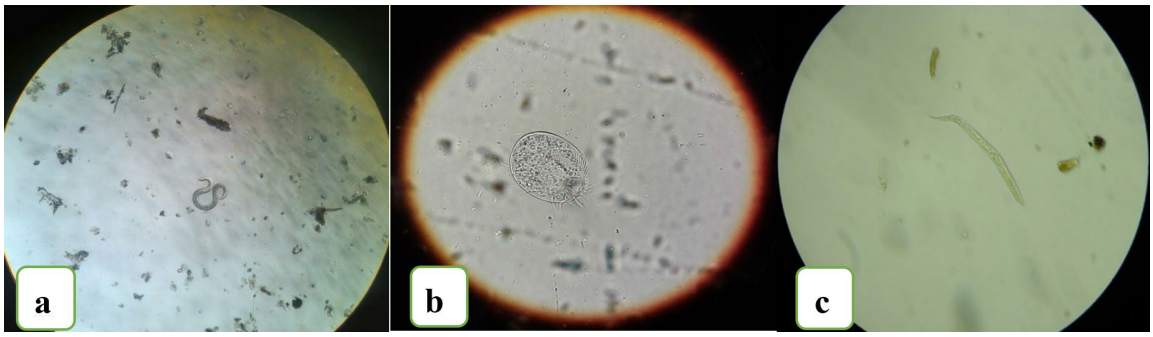

Figure 2. Microscopic slides of parasitic contamination isolated in salad vegetables. **a** Lettuce nematode; **b** Pepper trophozoite; **c** Mushroom nematode; (original picture, 400X)

the soil via contact with pets or the use of human/animal fertilizers. The average range of lettuce contamination in Iran in one study was 29.0% (15), which is higher than our results; also, they showed that the average leek contamination in Iran is 44.0%, making it the second most polluted vegetable (after scallions, 46.0%), which contrasts with our results. The reason for the low infection rate is probably due to our procedure for preparing vegetables before the examination process, mimicking how it is done traditionally in almost all houses and restaurants in Iran. For this purpose, the end of the leeks, which are the part that carries mud, was cut. However, our results did not show any relationship between parasitic contamination in unwashed vegetables and the type of vegetables. A similar correlation was found in Koya city, Iraq (37), and in the United Arab Emirates, where the prevalence of parasites was independent of the type of vegetable, with no significant relationship observed between vegetable type and contamination (38). In contrast to our study, in Ethiopia, a significant relationship was reported between the type of vegetables and parasitic contamination (29).

We also investigated the effect of seasons on parasitic contamination between unwashed vegetables. Our findings showed that there was no significant correlation between the seasons and the parasitic contamination of vegetables. However, the rate of contamination in our study was higher in spring and autumn (the relatively warm seasons). A similar correlation was observed in the study conducted in Koya City, Iraq (37). A study of Alexandria, Egypt, showed no significant difference between the number of contaminated samples in different seasons (2). however, contamination was notably higher in spring and summer compared to winter and autumn (2). Moreover, some studies reported a higher rate of parasites in fresh vegetables during warm seasons than cold seasons, which corresponds to our study (10, 39). It should be noted that

rainfall during the rainy season, which is exactly at the end of autumn and winter in Bushehr, minimizes the need for irrigation by other sources of water (5).

Moreover, our statistical data analysis revealed that nematode larvae, followed by trophozoites and cysts, were the most commonly detected types of parasites in the washed and unwashed vegetables. All of the detected parasites were animal or free-living parasites. The study in Golestan, Iran (36) reported that the nematode larvae were the most commonly counted parasites, and *Hymenolepis nana* eggs showed the lowest contamination rates. In other studies, nematode eggs (10, 15, 40), oocysts (2, 41), and cysts (42) were the most detected types of parasites. However, one study showed that the existence of some zoonotic parasites in vegetable salads has raised concerns in public health (13).

In addition, parasitic contamination in the present study showed a positive correlation with washing methods. It stated that washing only with tap water and the 3-step and 4-step washing methods have a positive effect on removing the parasites from vegetables compared to unwashed samples. While traditional washing reduces contamination to some extent, it does not eliminate the risk entirely and does not achieve the same level of effectiveness as the multi-step (3-step and 4-step) washing methods. Our results showed 3.0% parasitic contamination rate after washing using the traditional method, which is slightly higher than the 1.3% reported in a study in Shahrekord (10).

A few studies have been done in Iran to determine the efficacy of national or international washing methods. One study was done on parasitic contamination in Qazvin province, Iran (43). Other studies aimed to evaluate the efficiency of lettuce disinfection according to the official protocol in Iran (7), and another to determine the effect of the season and washing procedures to remove parasites from raw vegetables in Shahrekord, Iran (10). One study investigated the efficiency of different disinfectants on infection rates in fruits and vegetables available in the market of Kermanshah (4). Also, a 3-stage washing method was done on vegetables in Bursa, Turkey, in 2011 (44). All of these studies (4, 10, 43, 44) used calcium hypochlorite at a 200 ppm concentration as a disinfectant, but one study used benzalkonium chloride as a disinfectant, similar to our study (4). Moreover, in some of the mentioned studies, parasitic contamination was reported as zero percent in the standard washing method (with disinfectant) (10, 43, 44), which corresponds to our findings.

Soil is essential as a suitable substrate for parasitic worms to grow and reach the infectious stage of transmission (45). The plants that grow in the soil, including edible vegetables, are one way these parasites are transmitted (45). Although many of these parasites are animal parasites and not pathogenic to humans, they may have consequences for humans after they enter the body due to their incomplete development in the human body. In order to know the infection status of each region, even the infection levels of non-pathogenic parasites should be given sufficient attention, as the way these parasites are transmitted is similar to that of pathogenic parasites. In a study conducted on inhabitants of rural areas of Guilan Province, northern Iran, three species of non-pathogenic protozoa were identified: *Endolimax nana*, *Entamoeba coli*, and *Entamoeba hartmani* (46). We admit that there may be a limitation in our study, caused by the method used for detecting and counting the parasites, and it is possible that our results underestimate the actual infection rates. The technique used, i.e., direct observation using wet mount, may not have detected all of the characteristics of the parasite. Therefore, this is one of the limitations of this study. Another limitation is that we did not collect vegetable samples during summer; however, the spring and autumn seasons in Bushehr have climatic conditions similar to those of summer.

Conclusion

The result of this study demonstrates that raw vegetables sold in Bushehr markets are partially contaminated with parasites, especially nematodes, which may pose a health risk to consumers when consumed either unwashed or washed using traditional methods. For reducing parasitological disease and having a healthy diet, the standard vegetable washing method, i.e., the fourth method in this study based on the Ministry of Health's four-step washing guidelines, is recommended. These steps include: Step 1: rinsing with clean water; Step 2: washing with clean water and detergent; Step 3: soaking in disinfectant solution (a mixture of clean water and disinfectant); and Step 4: rinsing thoroughly with clean water (12). It is also necessary to conduct regular monitoring to ensure the proper implementation of the Ministry of Health's protocol for the disinfection of raw fruits and vegetables. Supervising the treatment of wastewater for crop irrigation and the use of human or animal fertilizers, preventing the presence of stray animals, and properly educating the members of society should be considered to reduce the risk of infection.

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Competing Interests

On behalf of the authors, the corresponding author states that there is no conflict of interest in this study.

Ethical Approval

This research was approved by the Ethics Committee of Bushehr University of Medical Sciences.

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