Research article

Journal of Human, Health and Halal Metrics; 2020, 1(2): 1-7
https://doi.org/10.30502/JHHHM.2020.247311.1023

Prevalence of parasitic contamination in vegetables distributed in Yazd city, center of Iran

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Submission: 15 July 2020 ***Revision***: 13 September 2020 ***Acceptance***: 26 September 2020

Abstract

Background and objective: Parasitic infections damage human health in different ways especially by gastrointestinal disorders. Several investigations have been done on determination of parasites in vegetables in the world. The aim of this study was to assess the rate of parasitic contamination in edible vegetables available in Yazd city (center of Iran) and suggestion of appropriate methods for prevention and control of the contamination.

Materials and methods: Four hundred samples of washed and unwashed vegetables were investigated by sedimentation and centrifugation method. The method was included to washing the vegetables by water and detergent and then rinsing several times. The sediments were centrifuged and finally stained with Lugol's iodine solution. Vegetables in the study were included to leek, parsley, coriander, dill, radishes, scallion, garden cress, basil, mint, and fenugreek.

Results and conclusion: Sixty fine out of 200 unwashed vegetables (32.5%) and three out of 200 washed vegetable (1.5%) were infected by parasites. The most contamination in the unwashed vegetables were related to terrestrial nematode, inhuman parasite eggs, and parasitic worm eggs, while their contamination dropped significantly in the washed vegetables. Among the all samples, leek and scallion were the most contaminated and radish had the least contamination. The highest level of contamination was observed in summer and the least level of contamination was observed in winter. In conclusion, parasitic contamination of the vegetables in Yazd was at moderate level. However, it is possible to prevent parasitic infection in humans by proper training of people in preparation and disinfection of vegetables.

Keywords: Food-borne diseases, nematode, parasite, vegetable

1. Introduction

Parasitic infections are of important health issues in the world, especially in tropical regions [1]. Iran is also involved in this area and it is necessary to monitor the pathogens and affecting factors [2]. Parasitic contamination affects human

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health in various ways; one of them is malnutrition [3]. Main parameters affecting the spread of human parasites are included to economic and social conditions of people and their knowledge, irrigation method in the farms, use of human and animal manure in agriculture, and climate changes [4,5].

Daily consumption of vegetables is recommended by dietitian (2–3 cups per day) because they are source of the functional ingredients of antioxidants, antimicrobials, and flavonoids [6-8]. However, they are a potential source of parasitic contamination. Consumption of vegetables, especially those unwashed, plays an important role in transmission of parasites. In this regard, reported cases of diseases have increased due to the consumption of parasites-infected vegetables in recent years [1]. Parasitic contaminations in vegetables have been reported in many parts of the world such as Ethiopia, Thailand, Italy, Egypt, Turkey, Saudi Arabia, Vietnam, Nigeria and India [9-16]. Similar studies have been carried out in Iran. According to the reports, prevalence of parasitic diseases in different places of Kerman, Kermanshah, Mazandaran, Kashan, Urmia, Yazd, Semnan, Ghaemshahr, Bandar Abbas, Ardebil, Khorramabad, and Qazvin was 47.2%, 59.13%, 21%, 46.9%, 22.5%, 61%, 13.7%, 8.4%, 48.4%, 27.7%, 52.7%, and 37.6%, respectively [17-23].

Due to a relatively high prevalence of parasitic infections, identification of the sources of infection and transmission as well as preventive actions are of the main priorities in food hygiene. Parasite contamination in human occurs in various ways of which the oral route is the most important and the most common [24]. As mentioned before, agricultural products of fruits and vegetables, especially the unwashed crops, play a major role [1,25]. In this regard, use of sewage in irrigation of vegetables is one of the main causes of parasitic infections in the products [26].

Various techniques have been developed in different parts of the world to detect parasitic contamination of vegetables. Identification of contamination at each region can help in their prevention and control. In Iran, studies have been done in the cities located at different geographical regions in this regard [4,27-29]. Results of the studies showed that consumption of unwashed and not disinfected vegetables always has a potential risk of parasitic infections. Due to the need of frequent evaluation of minimally processed food items such as vegetables, the aim of current study was to determine the type of parasites and the effect of preparation (washing) on decontamination of the vegetables harvested and distributed in Yazd. This makes an opportunity to find out the hygienic status of the vegetables to keep a desired level or make an urgent action in reducing the contamination, if exists.

2. Materials and methods

2.1. Samples and sampling method

Sampling was done among edible vegetables that are commonly used in most parts of the country. Four hundred samples of vegetables (200 washed and 200 unwashed) were prepared for analysis. Firstly, the city was divided into five regions (north, south, east, west and center), and one kg of each vegetable (leek, parsley, coriander, dill, radishes, scallion, garden cress, basil, mint and fenugreek) was randomly collected from the regions of which the washed samples were further prepared. All of the steps were repeated in four seasons of a year. Table 1 shows the frequency distribution of washed and unwashed vegetable samples.

Table 1- Frequency distribution of unwashed and washed vegetables collected from different regions of Yazd

	Unwashed		Washed	
Type	Number	Percent	Number	Percent
Leek	20	10	20	10
Parsley	20	10	20	10
Dill	20	10	20	10
Coriander	20	10	20	10
Radish	20	10	20	10
Scallion	20	10	20	10
Garden	20	10	20	10
Cress	20	10	20	10
Basil	20	10	20	10
Fenugreek	20	10	20	10
Total	200	100	200	100

2.2. Preparation of washed samples in the laboratory

172 grams of each sample was kept in a plastic container containing one liter of water and anionic detergent (two drops per liters) in order to reduce surface tension, and accelerate release of parasites' eggs, cysts, and larvae. The mixture was stored in the laboratory for 30 min. Then, samples were removed from the container and

washed under water pressure several times to move the parasites into the container. At the end, the remaining aqueous phase was left for about 24 h to help sedimentation of parasites' eggs, cysts, larvae, etc. [26].

2.3. Sample analysis

Method of Balarak et al. was used with some modifications [26]. For each sample, about 500 ml of the mixture containing sediments of the last step was transferred to a beaker in the laboratory and stored for 3-4 h. Then, the last sediments were transferred to several centrifugal tubes (four tubes for each sample and 10 ml of each sediment for each centrifugal tube). Centrifugation was done at 2000 rpm for 3 min. Then, precipitates of the four centrifugal tubes (from a same sample) were pooled in one centrifugal tube and was made to 10 ml by distilled water. The pooled solution was centrifuged again at 2000 rpm for 3 min. Two slides were prepared by using saline solution (0.85% w/v) and Lugol's iodine and screened by light microscope. In order to study the cryptosporidium oocyte, one drop was removed from the sediment and a thin slide was prepared. The slide was stained with a modified Ziehl-Neelsen method and then examined by a microscope under ×100 magnitude.

3. Results and discussion

Table 2 shows the frequency of each type of parasitic contamination in the unwashed samples. Based on the results, 65 out of 200 unwashed vegetables (32.5%) were infected at least by one type of the infectious particles.

In comparison, parasitic contaminations in unwashed vegetables in Tehran [30], Qom [26], Kerman [31], and Isfahan [19] have been reported as 65%, 31.5%, 34.2% and 13.76%, respectively. As observed, rate of contamination in the capital city of Tehran was significantly higher than the other cities such as Yazd. It might be due to the fact that Tehran is an over-crowded city and unpotable water used for irrigation of arable lands sometimes. Another study conducted in Yazd in 2017-2018 reported the parasitic contamination of unwashed vegetables as 25.7% that was lower than the amount observed in our study (32.5%) [32]. In the studies were done in Nigeria in 2007 and Turkey in 2005, the parasitic contamination of unwashed vegetables was reported as 36% [10] and 5.9% [11], respectively. These differences can be related to various factors such as geographical location and climate, health status of the farmers, methods of parasites' identification, type of irrigation, type of farming and animal husbandry, use of human or animal manure, parasitic infection in human, and handling of vegetables [25,26]. According to Table 2, scallion and leek had the highest contamination, which was consistent with the studies conducted in Qazvin and Kerman [4,33]. In accordance, in the study conducted in Saudi Arabia and Tokyo, the most contamination of parasites in leafy vegetables was reported in scallion [9].

Table 2- Type of parasite contamination at each unwashed vegetable collected from Yazd

Type	Terrestrial nematodes		Parasitic worm eggs		Inhuman parasite eggs	
	Number	Percent	Number	Percent	Number	Percent
Leek	6	15.79	3	37.5	2	10.53
Parsley	5	13.15	-	-	-	-
Radish	-	-	-	-	1	5.26
Mint	4	10.52	1	12.5	5	26.32
Basil	4	10.52	-	-	2	10.53
Dill	5	13.15	-	-	1	5.26
Coriander	5	13.15	-	-	-	-
Fenugreek	4	10.52	-	-	-	-
Scallion	3	7.89	3	37.5	5	26.32
Garden Cress	2	5.26	1	12.5	3	15.76
Total	38	100	8	100	19	100

The lowest level of contamination was observed in radish possibly because it is harvested once a year. Moreover, parasites are usually present on the leaves and the vegetables like radishes, in which the leaves are not commonly consumed, may have less potential of parasites contamination in human [26].

Type of contamination in the samples collected from different geographical regions is presented in Table 3. The highest level of contamination in the unwashed vegetables was observed in those of north region (15 cases) and the least contamination was detected in the samples of south. It might be due to the differences in population and different irrigation methods among the regions.

Table 3- Type of parasite contamination at unwashed vegetables collected from different regions of Yazd

Region	Terrestrial nematode		Parasitic worm eggs		Inhuman parasite eggs	
	Number	Percent	Number	Percent	Number	Percent
South	5	13.16	1	12.5	5	26.32
East	9	23.68	2	25	2	10.53
Center	7	18.42	2	25	3	15.78
West	8	21.05	2	25	4	21.05
North	9	23.69	1	12.5	5	26.32
Total	38	100	8	100	19	100

Table 4 differentiates the contamination type of all unwashed and washed vegetables. The highest level of contamination in both groups was related to terrestrial nematodes which might be due to the high terrestrial nematodes existed in soil. Importantly, only three washed samples infected by parasites showed that washing and disinfection could reduce and eliminate the contamination. As shown, the least contamination was associated with parasitic worm eggs which do not cause serious infections in human [34] but it indicates the samples' contact to animal manures or surface water that is hygienically important [12,26].

Table 4- Type of contaminations in unwashed and washed vegetables

Type	Unwashed		Washed	
	Number	Percent	Number	Percent
Terrestrial nematode	38	58.46	3	100
Parasitic worm eggs	8	12.31	0	0
Inhuman parasite eggs	19	29.23	0	0
Total	65	100	3	100

Table 5 shows the rate of contamination in the unwashed vegetables at different seasons. The highest and least level of contamination was observed in summer and winter, respectively. It was reported that excretion of parasite eggs to the environment by humans or animals is relatively high in warm seasons [35]. This finding strengths the hypothesis of using untreated wastewater for irrigation of the vegetables in Yazd which led to the higher rate of contamination in summer followed by spring. However, other factors may significantly affect the parasite contamination in agricultural crops. For example, both wild and domestic animals contribute directly to the contamination of vegetables and fencing the farms to control animals' movement inside the fields is a simple way of contamination control. In addition, construction of sanitary toilets in the fields and proper storage of fertilizers (especially long-term storage of compost) are the other feasible approaches. Avoiding the use of untreated animal manure as a natural fertilizer in the farms should also be considered. Nonetheless, consumers are strongly recommended to disinfect the vegetables other than washing alone to avoid contamination. Using anionic detergents to reduce surface tension is one available approach in this regard [35-40].

4. Conclusion

Inclusion of vegetables in daily food basket is necessary in healthy diet. Unfortunately, inappropriate farming, transportation, storage and in-

Table 5- Parasitic contamination of unwashed vegetables collected in Yazd at different seasons

Type	Spring		Summer		Autumn		Winter	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Terrestrial nematode	11	55	13	52	8	66.66	6	75
Parasitic worm eggs	3	15	3	12	2	16.67	-	-
Inhuman parasite eggs	6	30	9	36	2	16.67	2	25
Total	20	100	25	100	12	100	8	100

house preparation can make vegetables a source of human infection. Therefore, the all steps in handling of vegetables should be controlled and make sure that they are handled hygienically. Our investigation showed that parasitic contamination of the vegetables collected in Yazd was at moderate level. Proper training of people to disinfect the vegetables before use can be useful in prevention parasitic infection. Limitation of the current study was the samples collected from retailers around the city that was a challenge in tracing the suppliers. Therefore, planning a more throughout research to cover traceable samples is suggested.

5. Acknowledgement

The authors appreciate Iran Food and Drug Administration, Paramedical Faculty of Yazd University of Medical Sciences, and the students collaborated in conducting the research.

6. Conflict of interest

The authors are engaged in Yazd deputy of Iran Food and Drug Administration.

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