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Original Article

Evaluation of parasitic contamination in consuming vegetables in a city of Iran in 2011

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ABSTRACT

Aims: The aim of this study was to determine the prevalence of parasitic infection via vegetables consumed in Isfahan in 2011.

Materials and Methods: In this cross-sectional descriptive study, 120 samples of vegetables were collected from 12 sites in the vegetable supply of Isfahan. Then samples were washed carefully with the detergent, the solution condensed with the Sheeters flotation method and ether Ritchie method in case of detecting parasites with microscope. Statistical analysis was done with Excel and SPSS software.

Results: Among the samples, 48.4% were infected with pathogenic and nonpathogenic parasite eggs and larvae, and 51.6% of samples were not infected by parasite. Among the contaminated samples, 48.1% were nonpathogenic, and *Giardia* with a frequency of 0.4% was the only pathogenic parasite that observed.

Conclusion: The study showed that the percentage of parasites in raw vegetables was close to zero. However, the previous study in Isfahan city reported 13% contamination. The reduction in intestinal worm infections is probably the result of development of water and wastewater networks, improvement in public health and public knowledge about parasitic infections and different ways of its prevention.

Key words: Iran, Isfahan, parasitic contamination, vegetable

INTRODUCTION

Parasitic diseases are still one of the major complications especially in developing countries[1] in which parasites can infect the body through different routes including direct or indirect contact with soil and water. Besides, they can enter the body through the food chain, insects and seldom can be transmitted to the fetus.[2]

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Reports and statistical data about different diseases and percent of mortality because of parasitic infections throughout the world indicated a high risk of prevalence of parasitic infections in human societies.[3] The World Health Organization classified parasites as the sixth group among the most harmful causes of human infectious diseases.[4]

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However, it is illustrated that in most cases gastrointestinal infections won't be dangerous, but it can cause symptoms such as loss of appetite, weight loss, and weakness which may have a negative effect on the performance of individuals in the society and even cause to spend expenses for treating such infections.[5] *Ascaris lumbricoides*, *Trichuris trichiura*, *Trichostrongylus*, *Giardia lamblia*, *Tenia cyst*, *Hymenolepis*, and *Entamoeba histolytica* are examples of the pathogenic parasites that have been found in vegetables.[3-7]

Consumption of vegetables especially raw form of them consist a main part of Iranian food basket. While this dietary habit provides a significant percentage of vitamins and essential ingredients for the body, the parasitic infection risk always exists via vegetable ingestion. Vegetables can become contaminated with enteric bacterial, viral, and parasitic pathogens throughout the processes, from planting to consumption. Although, in recent years, chemical fertilizers are commonly used for agriculture but using manure as fertilizer in farms is still in common and can cause bacterial infections and parasitic contamination to the farms, vegetables and therefore to the human as the final consumers.[8]

Some previous studies have reported the rate of vegetable contamination with parasitic outputs from 1.94% to 68.3% in different regions of Iran.[9-13] Of course, some of them have reported the nonpathogenic parasites as well.[9,10]

Recent studies on the vegetables in Isfahan have considered the prevalence of parasites in agricultural lands.[6] In this study, the main criteria were to determine the relative abundance of parasitic and microbial contamination in vegetable supply centers. The aim of this study was to investigate the frequency and type of intestinal parasites in vegetables that consumed in Isfahan in 2011. Determining the prevalence of parasitic contamination among vegetables, illustrating the reasons of such contamination and addressing procedures for controlling and preventing parasitic diseases were other aims of this study.

MATERIALS AND METHODS

In this cross-sectional descriptive study, Isfahan city was divided into 6 parts, and 2 stores were selected accidentally in each area. Vegetables were purchased 3 times from each store. Sampling was occurred in the early morning hours. The vegetables selected for this study were selected in a manner that have a wide usage and were used in most parts of the country as raw form. These vegetables included leeks, mint, parsley, basil, radish, green onion, and lettuce. Each sample was about 150 g.

In the first stage, the vegetables were soaked inside a 10-L bucket of water in such a way that water covered surface of vegetables perfectly. In order to better isolation of the parasite from the vegetables, they stirred carefully; and after 10 min, the following procedures were used for separating parasite from the vegetables. In this experiment, three solutions were used for washing vegetable and separating parasites. About two-thirds of the samples were de-parasite by anionic detergent (dishwashing liquid), one-third of the samples were washed with Tween-80 as an anionic detergent which didn't fix the parasites and allowed us to observe movement of the parasite in the microscope. And finally for checking out accuracy, a washing de-parasite solution was made for a limited number of samples (300 ml of sodium dodecyl sulfate solution with 300 ml solution of 1% Tween and 240 ml of phosphate buffer solution mix well in 2160 ml of distilled water).[14]

After washing vegetables with any one of these three methods (in water containing dishwashing liquid, Tween-80, or washing solution), samples were stored in these solution for 15 min. Consequently in the last stage of washing sediments was separated, and vegetables were discarded. After 24 h rest, the supernatant water of the sediments was aspirated, the remaining solution (volume 100 ml) was poured into the test tubes. Accuracy was taken place by using four methods including: Including direct observation, formalin-ether, Telemann, and sucrose. At first for all method sediments were centrifuged for 4-5 min with 2000 rpm.

1. In direct observation method, several extensions of sediments were created and observed in the microscope.
2. In formalin - ether method, 5 ml of 10% formaldehyde was added to deposits and shacked. Next 3 ml ether was added to the above solution and the final centrifugation was performed in 1500 rpm for 5-10 min. A microscope was used for detection of parasitic infection through the sediments.
3. Telemann method was like formalin — ether method, but hydrochloric acid 15% was used instead of ether.
4. In the sucrose method, after samples centrifuged at 2000 rpm for 4-5 min, about 5 ml of normal saline was added to the precipitates. Then the suspension added to 20 ml of cooled sucrose (2M), and after centrifuged for 25 min at 2500 rpm the parasite forms were extracted from a the layer between water and sucrose. After 10 times dilution of the separated layer, it was centrifuged at 1500 rpm for 5-10 min. Finally, the residue was examined for the presence of parasites with a microscope.[7]

Consequently, after all the experiments finished, the data were collected and analyzed with Excel and SPSS software (IBM, Chicago, USA), and then the results were displayed as graphs and tables.

RESULTS

Among samples, 48.4% were contaminated with larva and pathogenic parasite cysts. 51.6% of samples did not reveal any contamination. The study shows that the only existent pathogenic parasite in vegetables was *G. lamblia* with a frequency of 0.4% and was found in just one sample

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during the research. Vegetables with contamination and those without contamination are shown in Table 1 with respect to the type of vegetables. It should be noted that green onion was the most contaminated sample and lettuce revealed the least contamination during the study. Figure 1 demonstrates the results of contamination among vegetables separately, and Figure 2 illustrates the parasitic contamination with respect to the type of present parasites in the vegetables regarding the type of them using a bar chart. The results also show the percent of pathogenic and nonpathogenic parasitic contaminations in sampling vegetables [Figure 3].

DISCUSSION

In this study, the only sample that was contaminated with *G. lamblia* was radish. It can be concluded that vegetables with bulb are the most contaminated ones because they are in direct contact with soil and irrigation water. If there was any contamination during planting and harvesting, these kinds of vegetables would be probably contaminated sooner and easier. In the previous studies that have been done on vegetables; the frequency of *G. lamblia* was relatively high. In Egypt, *Giardia* cysts founded about 6.7% in different vegetables.[15] Another study which was done in Saudi Arabia reported that *Giardia* cysts 31.6% in leafy vegetables examined[16] and in Iran it was about 10% in Isfahan,[5] 34.78% in Shahroud[17] and 10.4% in Tehran[3] which is an emphasis on low frequency of parasitic contamination in the studied vegetables in the present survey in Isfahan. This study in comparison to the other study that took place in Isfahan[6] indicates that the parasitic contaminations of vegetables have been decreased in this area and limited to a very low percent that cannot result in infectious diseases. And moreover, it was the same with the study of Beliani and Asl in Tehran that they found a few number of parasitic contamination in vegetables.[18] In a study in Iran, researchers tend to evaluate the efficiency of conventional disinfection methods on lettuce and finally they did not found any parasite cysts in the samples[19] and it shows that utilizing a perfect method for washing and disinfection of vegetables will omit most type of pathogenic and nonpathogenic microorganisms and parasites from vegetables. Hlavsa *et al*. illustrated in their investigation that the incidence of infectious diseases related to *G. lamblia* were decreased 50% in 2001 in comparison with 1998[20] that again reveals this fact that the promotion in public health and public knowledge about parasitic diseases and the ways of prevention will decrease the risk of parasitic diseases.

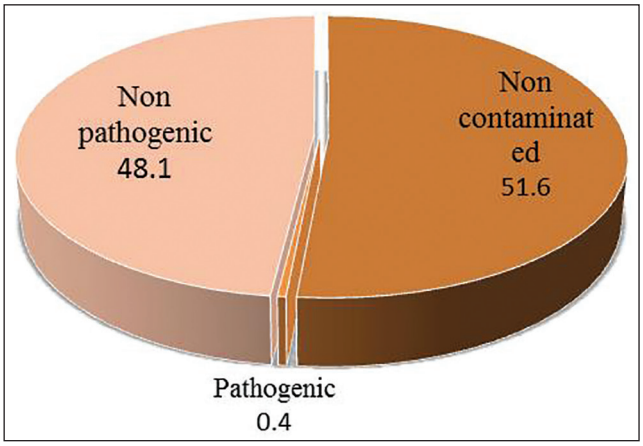

Figure 1: Percent of contaminated and noncontaminated samples and pathogenic condition

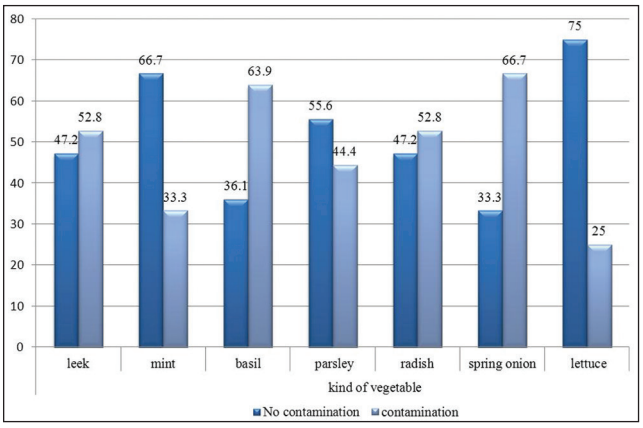

Figure 2: Frequency of parasitic contamination in vegetables with respect to the type of them

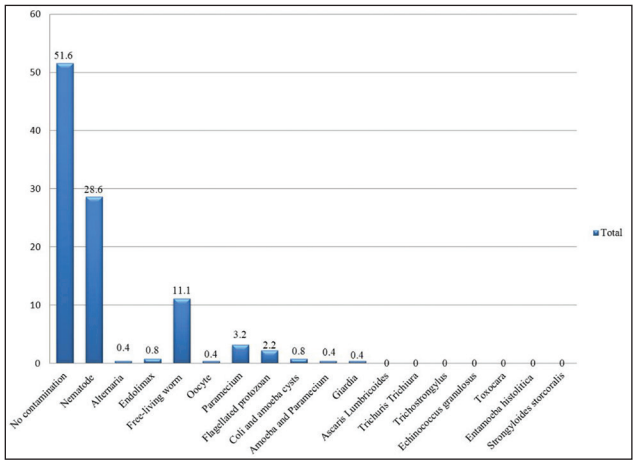

Figure 3: Frequency of parasitic contamination with respect to the type of parasites

Table 1: Percentage of contamination in consumable vegetables in Isfahan with respect to the type of vegetables

Condition	Vegetables							
	Leek	Mint	Basil	Parsley	Radish	Green onion	Lettuce	Total
Noncontaminated (%)	47.2	66.7	36.1	55.6	47.2	33.3	75	51.6
Contaminated (%)	52.8	33.3	63.9	44.4	52.8	66.7	25	48.4

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Comparison between the present study with recent surveys in different cities of Iran shows a better healthy condition of vegetables with respect to the parasitic contaminations that it probably illustrates the importance of improvement of public health in Isfahan and in agricultural methods from planting and harvesting to irrigation. The following studies are an emphasis on this fact; Homayouni and Khalagi analyzed 270 samples of vegetables which had a relatively high parasitic contamination with 41.3%.[3] Saki *et al*. reported 15.5% of parasitic contamination in 135 samples in Ahvaz,[1] and In Tabriz about 40% of markets vegetables and 76% of garden vegetables were contaminated with different parasites while prevalence of pathogenic parasites in vegetables of markets and gardens were 20 and 25%, respectively.[21]

Moreover, as it is indicated in Figure 2, the contamination with larva decreases and it is perhaps due to development of water distribution network and sewage collection system and improvement in agricultural methods.[19] Promotion in public health via public training of health programs was another reason for the reduction in intestinal infectious diseases.[22] There are some recommendations for controlling contamination and preventing transition of parasitic agents via vegetables. Followings are some of these recommendations:

- Sanitary disposal of sewage and human wastes,
- Avoiding usage of contaminated water for irrigation,
- Avoiding usage of sewage as fertilizer,
- Utilizing a perfect method for washing of vegetables before consumption,
- Public health training,[14]
- Construction of wall or hedge to prevent vegetables and farms to be contaminated by animals,[23]
- Utilizing of new forms of technical procedures in processing, decontamination, and packaging of vegetables.[24]

Nevertheless, in this study, the parasitic contamination of vegetables was the only factor that has been analyzed and more surveys on bacterial contamination are also recommended.

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