

The Prevalence of Parasitic Contamination of Vegetables Consumed in Malayer City, West of Iran, in 2014

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Abstract

Background: Transmission of infectious agents, such as parasites, is associated with consumption of raw vegetables. Thus, the health of vegetables reflects the health status of a region.

Objectives: Due to considerable parasitic contamination in Hamadan province and lack of information about health of vegetables in this region, this study was conducted in Malayer city, west of Iran.

Methods: This investigation was a cross-sectional study carried out on 383 samples of different vegetables including leek, parsley, coriander, radish, spring onion, tarragon, basil, mint, cress, and savory. The samples were randomly collected from 38 farms around Malayer city and subjected to parasitic contamination analysis using sedimentation and floatation methods.

Results: The results showed that 14.6% of the vegetable samples were contaminated with various pathogenic (5.2%) and non-pathogenic (9.4%) parasites including protozoan cyst (3.7%), worm eggs (3.9%), and free-living larvae (7%). *Giardia intestinalis* (1.3%) and *Entamoeba coli* (2.3%) were the only protozoa that were detected in the samples. Frequencies of worm egg contamination were 1.6% for *Taenia/Echinococcus* spp., 0.5% for *Dicrocoelium dendriticum*, 0.8% for *Toxocara* spp., 0.5% for *Hymenolepis nana*, 0.3% for *Trichostrongylus* spp., and 0.3% for *Fasciola* spp. Leek was the most contaminated vegetable (31.7%), although there was no contamination in tarragon ($P < 0.001$). Significant relationships were observed between parasitic contamination and fertilizer ($P = 0.018$) and water consumption ($P < 0.001$) used in the farm vegetables.

Conclusions: The results demonstrate the potential role of raw vegetables consumption in the transmission of parasitic infections in the area. Therefore, it is recommended for some necessary hygienic measures to be applied to increase the public health of the community.

Keywords: Food contamination, Iran, Parasites, Vegetables

1. Background

Many societies, especially developing countries, consider intestinal parasitic infections as one of the major public health problems. These infections can cause serious medical and public health concerns, such as malnutrition and growth retardation of children, which can lead to a significant number of morbidity and mortality annually (1, 2). Millions of people suffer from parasitic infections in developing countries because of a shortage of personal health services and poor sanitation (3, 4). The prevalence of intestinal parasites is associated with the consumption of raw vegetables in these countries (5, 6). Food and water-borne diseases are a major health issue in developing and even developed world (7). Fresh vegetables and fruits are an important source of nutrients and bioactive non-nutrient plant compounds, including vitamins, minerals, phytochemicals, especially antioxidants, and dietary

fibers (8, 9). Daily use of fruits and vegetables can reduce the risk of chronic diseases, such as cardiovascular disease, cancer, age-related decrease, and improve the function of the digestive system. Thus, nutritionists recommend eating fresh fruits and vegetables in daily diet (10). In order to preserve the flavor and nutrients of vegetables, they are usually consumed raw, which can increase the risk of infectious microbial diseases (3, 11). Consumption of raw vegetables is the most important way of transmission of intestinal parasites and is often considered as a major concern of public health. Protozoan cysts and worm eggs and larvae survive and develop in moist soil and environments of farm vegetables. These conditions are necessary for continuation of soil-transmitted parasite life cycle (7, 12). Vegetables can become contaminated mainly through irrigation and fertilization with untreated sewage water and animal manure, respectively, and may also occur during unsanitary post-harvest processing and marketing

(3, 6, 13, 14).

2. Objectives

Because of little information about potential microbial hazards of agricultural products in Hamadan province, this study was conducted to evaluate parasitic contamination of green leafy vegetables in Malayer city, West of Iran.

3. Methods

3.1. The Study Area

This cross-sectional study was performed from July to October 2014 in Malayer, the second largest city of a province with a population of over 287,982 (15) and an area of 3210 square kilometers. The study area lies between 48° 49' longitude east and 34° 17' latitude north with 1725 meters of altitude above sea level. The mean temperature and annual precipitation of the region are 13.2°C and 300 millimeters with about 50 freezing degree days per year (Available on site: <http://www.iranhydrology.net/meteo.asp>).

3.2. Sampling and Sample Preparation Procedure

Thirty-eight out of 197 farm vegetables were selected and a total of 383 samples were collected accidentally for parasitic contamination analysis. The samples comprised of ten types of vegetables, including leek, parsley, basil, tarragon, savory, coriander, mint, spring onion, cress, and radish. Each sample was gathered separately in a clean nylon bag and, after labeling and recording its characteristics, it was transferred to the parasitology research laboratory of Hamadan University of Medical Sciences. After cleaning and removing soil and coarse particles of the sample, 250 g of each individual sample was immersed in 1 L of normal saline solution containing detergent (1% sodium dodecyl sulfate and 1% Tween 80), followed by continuous shaking for 1 hour. The vegetable samples were removed and the remaining wash water was left to sediment about 12 hours. The supernatant water was discarded and the sediment was passed through sieve No 40 (425 μ m), in order to eliminate large particles and detect larvae and free-living nematodes; the final 50-mL volume was then centrifuged at 500 g for 15 minutes (16). Finally, the sediment was examined by direct wet mount and zinc sulphate flotation method, according to the following procedure, after preparation of direct smear, the samples were scanned by a light microscope using low (100 \times) and high (400 \times) magnifications. The test was repeated several times for each sample and the remaining sediment was flooded with zinc

sulphate solution centrifuged at 250 g for 5 minutes. After centrifugation, convex meniscus on the top of the tube was created by adding flotation solution and covered by a clean coverslip. After 30 minutes, the coverslip was cautiously removed and subjected to direct wet mount examination, as described above (3). Protozoan cysts identified by Lugol's iodine and trichrome staining and also morphological characteristics were applied to differentiate larvae and eggs of helminths.

3.3. Data Analysis

Chi-squared (χ^2) and Fisher's exact test were used to evaluate the relationship between parasitic contamination and epidemiological factors studied with a probability (P) value of < 0.05, by using the SPSS statistical software, version 16.

4. Results

Out of 383 vegetable samples, 56 (14.6%) were contaminated with various pathogenic and non-pathogenic parasites including protozoan cysts (3.7%), worm eggs (3.9%), and free-living larvae (7%). The frequencies of pathogenic and non-pathogenic parasitic agents were 5.2% and 9.4%, respectively. The highest pollution of vegetables was associated with free-living larvae (7%) and the lowest belonged to *Fasciola* spp. (0.3%) and *Trichostrongylus* spp. (0.3%).

Frequency of parasitic contamination in different types of vegetables is indicated in Table 1. The highest prevalence of parasitic contamination was detected in leek (31.7%), while no contamination was observed in tarragon ($P < 0.001$).

In this study, 28 of the vegetable farms were irrigated with well water and 10 of them were irrigated with surface or waste-water. Animal and chemical manure were used to fertilize 33 and 5 of the considered agricultural lands, respectively.

Table 2 shows distribution of the parasitic contamination of vegetables, based on type of water and fertilizer used in agricultural farms. Nearly all of the contaminated vegetables (96.4%) belonged to the farmlands that are fertilized with animal manure. There were significant correlations between parasitic contamination and fertilizer ($P = 0.018$) and water consumption ($P < 0.001$) used for growing vegetables.

5. Discussion

Recommendation to use fresh fruit and vegetables is one effective way to promote health in the society (10). Vegetables may be contaminated with various enteric

Table 1. Distribution of Protozoan Cysts, Larvae, and Eggs of Helminths in Vegetables Used for Raw Consumption in Malayer City, Iran^a

Type	Vegetables Number	Pathogenic Parasites						Non-Pathogenic Parasites			Total
		<i>G. intestinalis</i> spp.	<i>D. dendriticum</i> spp.	<i>Toxocara</i> spp.	<i>H. nana</i> spp.	<i>Taenia</i> spp.	<i>Trichostrongylus</i> spp.	<i>Fasciola</i> spp.	<i>E. coli</i>	FL	
Radish	38	2 (5.3)	1 (2.6)	0 (0)	1 (2.6)	0 (0)	0 (0)	0 (0)	1 (2.6)	3 (7.9)	8 (21.1)
Cress	38	1 (2.6)	0 (0)	0 (0)	0 (0)	1 (2.6)	0 (0)	0 (0)	1 (2.6)	6 (15.8)	9 (23.7)
Spring onion	38	1 (2.6)	0 (0)	2 (5.3)	1 (2.6)	1 (2.6)	0 (0)	0 (0)	0 (0)	4 (10.5)	10 (26.3)
Savory	38	1 (2.6)	0 (0)	0 (0)	0 (0)	1 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.6)
Mint	38	0 (0)	0 (0)	1 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (5.3)	3 (7.9)
Basil	38	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.6)	0 (0)	0 (0)	1 (2.6)	2 (5.3)	4 (10.5)
Parsley	38	0 (0)	1 (2.6)	0 (0)	0 (0)	1 (2.6)	0 (0)	0 (0)	2 (5.3)	0 (0)	4 (10.5)
Tarragon	38	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Coriander	38	1 (2.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (7.9)	4 (10.5)
Leek	41	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.4)	1 (2.4)	1 (2.4)	3 (7.3)	7 (17.1)	13 (31.7)
Total	383	5 (1.3)	2 (0.5)	3 (0.8)	2 (0.5)	6 (1.6)	1 (0.3)	1 (0.3)	9 (2.3)	27 (7)	56 (14.6)

Abbreviation: FL, free-living larvae.

^aValues are expressed as No. (%).**Table 2.** Distribution of Parasitic Contaminations in Vegetables Grown in Farms With Different Types of Water and Fertilizer Consumption in Malayer City, Iran^a

Water and Fertilizer Consumption	Parasitic Contamination		
	Positive	Negative	Total
Well water	23 (8.2)	259 (91.8)	282 (100)
Surface and waste water	33 (32.7)	68 (67.3)	101 (100)
Animal manure	54 (16.2)	279 (83.8)	333 (100)
Chemical manure	2 (4)	48 (96)	50 (100)

^aValues are expressed as No. (%).

pathogens, including bacteria, viruses, and parasites during the cultivation and marketing process (3, 6, 13, 14). Recovery of parasitic agents from raw vegetables can be considered as a health indicator in the community and indicate the link between public health and agricultural policies and programs.

The present study showed that 14.6% of green leafy vegetables collected from Malayer city lands were contaminated with parasites. In comparison with other studies, the 14.6% prevalence of parasitic infestation is relatively moderate. There are different reports about the prevalence of parasitic contamination of raw consumed vegetables in Iran and other regions of the world such as Kenya (65.5%) (17), Libya (58%) (6), Egypt (31.7%) (14), Nigeria (3.5%) (5), Iraq (49.79%) (18), and Turkey (6.3%) (19). Klapac and Borecka reported that the rate of geohelminths eggs on vegetables and fruits from conventional and organic farms was 34.7% and 18.9%, respectively, in south-eastern Poland (20). The other reports from different areas of Iran are 52.7% in Khorramabad (16), 46.5% in Amol (7), 43.7% in southern Iran

(21), and 71% in Ardebil (22). The various prevalence rates of contamination may be due to date of studies, the application of different examination methods, and different epidemiological factors such as geographical and climatic conditions of the region, type of water and fertilizer used for growing vegetables, contamination after harvest, and other influential factors.

In this study, the highest prevalence of contamination belonged to free-living larvae (7%), because of the presence of free-living and plant-parasitic nematodes in the terrestrial environment. This finding is in accordance with the study of parasitic contamination of consumed vegetables in Khorramabad and Amol reported by Ezatpour et al. (16) and Siyatpanah et al. (7). Leek with 31.7% contamination was the most infested vegetable in the present study that agreed with some previous reports (15, 18, 23). High contamination in leek seems to be due to direct contact of its leaves with soil.

Considerably, *Ascaris lumbricoides* and other human intestinal nematode eggs were not detected in this study. However until a decade ago, human intestinal nematodes, especially *Ascaris lumbricoides*, were prevalent parasites in Hamadan province. Fallah et al. reported prevalence and moderate intensity of ascariasis as 19.5 and 59.3%, respectively, in Hamadan city, during year 2003 (24). Recently, some conducted studies in the area showed significant reduction in the parasites prevalence such as human intestinal parasitic survey in Hamadan province and among rural inhabitants of Hamadan city, as indicated by the studies of Fallah et al. in 2014 (unpublished data) and Jafari et al. (25) in 2012, respectively. A similar study was simultaneously conducted on farm vegetables of Asadabad, one of the cities of Hamadan province. The findings of

this study were close to those of the present study. The parasitic contaminants of vegetables were *Entamoeba coli* (2.6%), *Giardia intestinalis* (1.6%), *Toxocara* spp. (0.8%), *Fasciola* spp. (0.5%), *Dicrocoelium dendriticum* (0.3%), and *Taenia/Echinococcus* spp. (0.3%) (in press). Improvements in public health conditions and implementation of control measures against intestinal nematodes, particularly mass chemotherapy (26), caused high reduction in intestinal parasites, especially intestinal worms, in this area.

Giardia intestinalis cyst was observed in 1.3% of the vegetable samples, which is lower than other reports from Khorramabad (1.1% in winter and 5.8% in spring) (16), Ardabil (10% in imported and 18% in native vegetables) (22), Amol (22.5%) (7), Zahedan (8.1%) (12), and southern Iran (11%) (21). Cyst of *Entamoeba coli*, non-pathogenic intestinal protozoa, was the second prevalent parasite with a frequency of 2.3%. Detection of *E. coli* cysts in the samples can be considered as an indicator of fecal contamination of the vegetables.

Eggs of *Taenia saginata*, *T. solium*, and *Echinococcus granulosus* are morphologically indistinguishable from each other using microscopic or other parasitological techniques. In this survey, *Taenia/Echinococcus* spp. eggs were found in 1.6% of the samples tested for detection of parasite agents. In a few studies conducted by Ezatpour et al. in Khorramabad (16) and Siyatpanah et al. (7) in Amol, contamination of vegetable samples with *Taenia* spp. was not reported, whereas other studies in Iran reported contamination with *Taenia* spp. in examined vegetables (12, 13, 21-23). In the present study, the suspected cestode eggs probably related to *Echinococcus granulosus* because prevalence of human taeniasis is rare and instead prevalence of echinococcosis in stray dogs and livestock hydatidosis are relatively common in the study area (27, 28), although animal taeniasis should not go unnoticed. Hydatidosis is one of the serious zoonotic parasitic diseases that involves human and farm animals. In Iran, it is estimated that cost of human hydatidosis is annually US \$93.39 million with 1295 cases of surgical hydatidosis and an estimated 635 232 asymptomatic individuals (29). The eggs were dispersed in the environment by dogs or other wild canids, as definitive hosts. Infection of human, as an accidental intermediate host, frequently occurs through ingestion of the eggs during eating and drinking of contaminated foods and water. Consumption of infested vegetables by taeniid eggs increases the risk of human cystic echinococcosis and it is a great concern to consumers.

Ingestion of *Toxocara* spp. eggs can cause toxocariasis, visceral, and ocular larva migrans, in young children. Dogs, cats, and other wild canids and felids are responsible for the spreading of eggs in the environment (21). The results showed 0.8% contamination with *Toxocara* spp. eggs

in the vegetable samples. This report was in accordance with some previous reports (6, 7, 12, 13, 20, 21) and in contrary to some others (16-18, 22). High frequency of *Toxocara* spp. (48.3%) was reported by Maikai et al. from Nigeria (3).

A few zoonotic intestinal helminths, including *Dicrocoelium dendriticum*, *Fasciola* and *Trichostrongylus* spp. were detected in this study. Prevalence of the parasites in the environment chiefly depends on the rate of the infection in livestock and use of animal fertilizer in farm vegetables.

In conclusion, although this study and other evidences show that the prevalence of human intestinal parasitic infection is decreasing in the Hamadan province, yet the findings indicate that vegetable consumers in Malayer city are at risk of infection with parasitic protozoa and zoonotic helminth parasites. Thus, it is essential for some hygiene measures to be considered, including proper washing and disinfection of raw vegetables before consumption, implementation of sanitation practices, agricultural reform, and other health interventions that can be effective in the control of intestinal infections and public health promotion.

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Footnotes

Authors' Contribution: Khadije Rahmati contributed to the sample collection and experiments; Mohammad Falah and Amir Hossein Maghsood contributed to the study design and manuscript proofreading; Tayebeh Shamsi-Ehsan contributed to the experiments; Mohammad Martini contributed to the study design, experiments, and manuscript preparation.

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