



Parasitic Contamination of Raw Vegetables Consumed in Hamadan, West of Iran During 2017-2018

Monir Taherimoghaddam¹, Faeze Foroughi-Parvar^{2*}, Manizheh Kashinahanji², Mohammad Matini²

¹Student Research Committee, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

²Department of Medical Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

***Corresponding author:**

Faeze Foroughi-Parvar,
Department of Medical
Parasitology and Mycology,
School of Medicine, Hamadan
University of Medical
Sciences, Shahid Fahmideh
Street, Hamadan, Iran.
Tel: +989397307253;
Email:
forooghparvar@yahoo.com

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Abstract

Background: Healthy diets including fruits and vegetables reduce the risk of chronic diseases. This study was aimed at detecting parasitic contamination caused by consuming raw vegetables sold in Hamadan, west of Iran.

Methods: A total of 380 vegetable samples consisting of parsley, coriander, basil, savory, mint, cress, tarragon, leek, radish and spring onion were evaluated microscopically after conducting sedimentation and flotation from April 2017 to March 2018.

Results: Parasites were detected in 95 out of 380 of vegetable samples (25%). The organisms identified included *Entamoeba coli* (8.15%), *Ascaris lumbricoides* (7.1%), *Chilomastix mesnili* (5%), and *Giardia lamblia* (1.5%), *Blastocystis hominis* (0.78%), *Toxocara canis* (0.26%) and *Hymenolepis nana* (0.26%). High level of contamination was obtained for parsley (5.52%) while radish was the least contaminated (0.78%). Vegetable contamination was higher in spring and summer (20.6%) ($P < 0.0001$). Samples collected from vegetable fields irrigated by well water supply showed low parasitic contamination (28.5%) compared to those from farms irrigated by surface or sewage water (91%) ($P < 0.0001$).

Conclusions: The results emphasize the potential role of raw vegetables consumption in transmission of pathogenic organisms. Besides, it was highlighted that irrigation of vegetables by surface water could be the source of vegetable contamination with human intestinal parasites.

Keywords: Food Contamination, Parasites, Vegetables, Iran

Background

Healthy diet including fruits and vegetables reduces the risk of chronic disease such as heart disease, cancer, stroke and diabetes. A broad range of nutrients, phytochemicals, vitamins and minerals are provided through adhering to the diets containing the fruits and vegetables (1). Since the vegetables are served uncooked, without any processing, the contamination of these products seems very important. They can transfer pathogens from planting through consuming process (2). Vegetables will be contaminated with viral, bacterial or parasitological pathogens from different sources, such as human feces and manure that are widely applied as soil fertilizers. Untreated sewage is also used as a source of agricultural water in some regions (3). Foodborne disorders have attracted the attention of public health authorities across the world, as the World Health Organization (WHO) has reported 31 bacteria, viruses, parasites and toxins as the causes of food borne diseases particularly in low socio-economic regions in the world (4). Enteric outbreaks have been reported following consumption of tomatoes, spinach, lettuce and seed sprouts (3). Hamadan province is located in a mountainous region

in west of Iran. Vegetables are provided by local farmers in spring and summer but in autumn and winter vegetables are imported from relatively hot regions such as Khuzestan province due to the cold weather of Hamadan province.

Objectives

The present study was conducted to investigate the prevalence of parasitic contamination caused by consumption of raw vegetables as potential source of parasite dissemination.

Material and Methods

Sampling

This study was carried out in Hamadan, west of Iran. The city is located between 34°8' latitude north and 48°5' longitude east, and is 1810 m above the sea level, with a population of 676105 according to 2016 Iranian Population and Housing Census (5). This study was carried out from April 2017 to March 2018. For this purpose, first sampling was done during spring and summer and the second sampling was carried out over the autumn and winter. Samples included parsley, coriander, basil, savory, mint, cress, tarragon, leek, radish and, spring onion which

were purchased from different farms or markets.

Sample Preparation and Parasite Detection

A total of 380 vegetable samples were collected from urban and rural areas of Hamadan. Two hundred to 250 g of each vegetables was collected separately in zipped bags. Soil and coarse particles were removed from the samples before they were transferred to Parasitology Research Laboratory of Hamadan University of Medical Sciences. Procedures were followed according to those used in previous studies (4,6-9). Briefly, each weighed sample was left in 1 L of tap water containing 2% of detergent for 1 hour during which the samples were shaken constantly. The vegetable was picked up slowly and the remained water was left for 12 hours. Then the supernatant liquid was discarded and the sediment was passed through the sieve no. 40 (425 µm) to eliminate large particles and to monitor the larva or free living nematodes. The sample at the final volume of 50 mL was centrifuged at 500 rpm for 15 minutes, a drop of sediment was placed on a clean glass slide under microscopy for detection of eggs, larva or protozoan cysts (10). The zinc sulfate flotation tests were also done. Protozoan cysts were identified using Lugol's iodine following Trichrome staining technique for accurate differentiation (9).

Data Analysis

Statistical analysis was carried out using chi-square test by the SPSS software version 24. Potential differences in contamination among different seasons, samples and irrigation systems were considered significant at $P < 0.05$.

Results

Parasites were detected in 95 out of 380 samples. Twenty-five percent of samples were found to be contaminated. The most prevalent parasite was *Entamoeba coli* that was detected in 8.15% of samples, followed by *Ascaris*

lumbricoides and *Chilomastix mesnili*. The highest rate of contamination was reported in parsley (5.52%) and the lowest in radish (0.78%). Distribution of parasites based on types of vegetables is listed in Table 1.

Based on seasonal variation, parasitic contamination rate was highest in spring (8.67%) and lowest in winter (0%). Vegetables showed more contamination in spring and summer than in autumn and winter ($P < 0.0001$). The results showed that 14.47% of protozoan cysts and 1.8% of worm eggs were identified in vegetables collected in spring and summer. The contamination results showed 3.9% of protozoan cysts and 3.6% of egg worms in autumn and winter.

Regarding irrigation procedures in farms, 31.5% of vegetables were cultivated in farms irrigated by surface or sewage waters and 55.2% of vegetables in farms irrigated by well water supply. The analysis revealed that in 11 of 12 farms (91%) irrigated by surface or sewage water sources, vegetables with at least one parasitic contamination had been produced whereas 6 of 21 (28.5%) the products cultivated in farms irrigated by well water supply were contaminated with at least one parasite. Samples collected from farms irrigated by well water supply showed the lowest contamination compared to those from farms irrigated by surface or sewage water ($P < 0.0001$).

Discussion

The consumption of fresh fruits and vegetables is considered as a determinant of healthy diet. Despite the advantages of raw vegetables, they are regarded as a potential source of microbial transmission to human (7). Although dietitians advise eating raw vegetables in order to remain healthy, the hygienic way of using them is highlighted more than ever (11). Isolation of parasites from vegetables is characteristic for all processes involved in planting, irrigation, growing and harvesting (12,13). In the present study, total parasite

Table 1. Distribution of Protozoan Cysts, Larvae and Helminth Egg in Raw Vegetable

	Samples Tested No.	Parasites								Total
		<i>Ascaris lumbricoides</i> Ova, No. (%)	<i>Toxocara</i> Ova, No. (%)	<i>Hymenolepis nana</i> Ova, No. (%)	<i>Entamoeba Coli</i> Cyst, No. (%)	<i>Giardia lamblia</i> Cyst, No. (%)	<i>Chilomastix mesnili</i> Cyst, No. (%)	<i>Iodamoeba butschlii</i> Cyst, No. (%)	<i>Blastocystis hominis</i> Cyst, No. (%)	
Parsley	38	2 (0.52)	-	-	6 (1.57)	-	8 (2.1)	3 (0.78)	2 (0.52)	21
Coriander	38	2 (0.52)	-	-	2 (0.52)	4 (1)	-	-	-	8
Basil	38	1 (0.26)	-	-	1 (0.26)	-	2 (0.52)	-	-	4
Savory	38	3 (0.78)	-	-	2 (0.52)	-	4 (1)	1 (0.26)	-	10
Mint	38	1 (0.26)	1 (0.26)	-	7 (1.84)	2 (0.52)	3 (0.78)	1 (0.26)	-	15
Cress	38	4 (1)	-	-	3 (0.78)	-	4 (1)	-	-	11
Tarragon	38	4 (1)	-	-	4 (1)	-	1 (0.26)	-	-	9
Leek	38	3 (0.78)	-	-	6 (1.57)	-	-	-	1 (0.26)	10
Radish	38	3 (0.78)	-	-	-	-	-	-	-	3
Spring onion	38	-	-	1 (0.26)	2 (0.52)	-	1 (0.26)	-	-	4
Total	380	23 (6)	1 (0.26)	1 (0.26)	33 (8.68)	6 (1.57)	23 (6)	4 (1)	3 (0.78)	95

contamination of vegetables was estimated at 25%. This result is consistent with studies of Alhabbal in Syria (31.38%) (13), Fallah et al in Shahrekord, southwest Iran (32.6%) (14) and Ishaku et al in Nigeria (20%) (15). In to recent studies conducted near Hamadan, comparatively lower contamination rates have been recorded; for example, Rahmati et al (9) and Matini et al (6) reported that 14.6% and 8.4% of raw vegetables were contaminated, respectively. Weather condition, irrigation system, type of fertilizer, the sewage system and the rate of regional parasitic infection are effective on the rates of contamination in these areas. In this study, *Eantamoeba coli* was found as the most predominant parasite in all samples (2.6%). This result is in agreement with the finding of Matini et al that reported *E. coli* as the most frequently detected parasite with the prevalence of 8.15% (6). In the study of Gabre and Shakir (16) in Saudi Arabia, *E. coli* was found as the most frequent parasite (23.9%) and Daryani et al (17) reported that 10% of vegetables in Ardabil were contaminated with *E. coli*. Although *E. coli* is not regarded as a parasite, the existence of this microorganism in fruits or vegetables is an indicator of human feces influx into fresh vegetables (8,17,18).

Ascaris lumbricoides was the second leading common parasite found in vegetables (7.1%). Studies conducted in Shahrekord, Iran (14), India (19) and Ethiopia (20) showed the prevalence of *A. lumbricoides* were 14.1%, 36% and 20.83%, respectively. *A. lumbricoides* is one of the most important soil transmitted parasites whose prevalence has recently been reported to range from 1.5% to 53.2% in the world (13). Though the incidence of ascariasis has decreased in recent years, vegetable contamination by this parasite may indicate the potential risk of its re-emergence in the region (6). Contamination of vegetables by the helminth eggs might be associated with reusing raw wastewater in irrigation, using human fertilizers and post-harvest handling. In addition, the helminth eggs including *Ascaris ova* are very resistant to environmental factors and are transmitted to consumers easily (19).

In the current study, parsley was the most common contaminated vegetable (5.52%). Alhabbal (13) in Syria and Gharavi et al (21) in Iran observed the predominance of contaminated parsley in the vegetables. This could be attributed to dense foliage, rough surface and deep grooves of this plant, protecting protozoa cysts and helminth eggs against environmental threats.

Regarding seasonal variability, contamination of vegetable samples was highest in spring (8.6%) and summer (7.6%) and lowest in autumn (6.7%) and winter (0) ($P < 0.005$). Previous studies have confirmed the higher parasitic contamination and parasitic diversity of vegetables during warm seasons (14,22,23). In Hamadan, water supplies are provided by the river water, untreated waste water or surface water to be used for irrigation of vegetables cultivated throughout spring and summer, while

during autumn and winter, vegetables are imported mostly from the farms of Khuzestan, southwest of Iran, which are irrigated by rain or underground water. Furthermore, increased number of parasites in vegetables during warm seasons might be due to the increase of the temperature intensifying the excretion of parasites by humans or animals (24).

Surface water can be contaminated with human or animal feces in the path and the resulting contamination may transmit the helminth ova or protozoan cysts to vegetables. Bekele found out the determinant role of water supply in contamination of vegetables (20). In our study, 31.5% of vegetable samples had been cultivated in farms irrigated by surface or sewage water, while 55.2% of vegetables were obtained from farms irrigated by well water. Our investigations showed that 28.5% and 91% of products irrigated by, respectively, well water and surface water were contaminated with at least one parasite species. A significant difference was observed between parasitic contamination and irrigation system ($P < 0.005$).

Conclusions

The result of present study indicated that raw vegetables could be a potential source of parasitic infection. Regarding local preference for raw vegetables, the way of proper washing of vegetables should be educated in order to avoid the adverse health effects of intestinal parasites. It is necessary to improve the knowledge of local farmers about disadvantages of using surface or waste water for irrigation of vegetables.

Ethical Approval

The present study was approved by Ethics Committee of Hamadan University of Medical Sciences (Ethics Committee reference number: IR.UMSHA.REC.1396.131).

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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