



Parasitic Contamination of Raw Vegetables in Zanjan Markets, Iran

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ABSTRACT

Background: Complex surface of vegetables facilitate attachment and transmission of several pathogens. No previous study has been conducted in survey of parasitic contamination of vegetables in Zanjan. This study aimed to detect the parasitic contamination in common raw vegetables in Zanjan markets.

Methods: A total of 352 raw vegetable samples, including leek, parsley, basil, mint, radish, cress and dill were collected from grocery stores using cluster sampling in different regions of the city during 2014. The edible parts of vegetables were separated and immersed in normal saline solution. Floating vegetables were removed and the solution was allowed to sediment at room temperature for 24 hours. The pellet was examined following sedimentation and floatation methods.

Results: Various Organisms were detected in 54% (190) of the 352 samples, but only 2.8% of samples had pathogenic parasites including; *Trichostrongylus* eggs (3), Hookworm eggs (2), *Eimeria* oocysts (2), *Sarcocystis* oocyst (1), *Strongyloides* larvae (1), and *Fasciola* eggs (1). The contamination rate of vegetables was highest (90.4%) in the fall ($p < 0.05$).

Conclusion: Vegetable contamination with parasitic organisms in this area was low, maybe due to irrigation of vegetables with sources other than sewage water, but it is still necessary to improve sanitary conditions of vegetables.

1. Introduction

Fresh vegetables are major source of vitamins, fiber, minerals and have a great value in people's diet [1, 2]. Eating raw vegetables without proper washing is a major route of transmission of soil and vegetable-transmitted parasites. Vegetables can be contaminated during cultivation, harvest, transport, distribution and even at home. In addition, contaminated vegetables can transmit the infectious agents to workplaces, containers, and people who handle them.

The possibility of food and vegetable-borne infections has increased with changing life styles, people's inclination to eat at restaurants, the presence of vendors, aged population, and malnutrition the increase of immunocompromised individuals has also led to an increase in vulnerable population. In such conditions, the parasites that are not normally harmful for healthy and young individuals will become a public health threat and pathogenic agents.

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Vegetables can transmit cysts and oocysts of protozoa such as *Giardia*, *Entamoeba*, *Toxoplasma*, and *Isospora* as well as eggs and larva of helminthes such as *Hymenolepis*, *Taenia*, *Fasciola*, *Trichuris*, *Trichostrongylus*, *Strongyloides*, and hookworms [3]. Soil transmitted and food-borne parasites are endemic in Iran and other developing countries.

Although, in recent years, the prevalence of some parasites, especially intestinal nematodes have significantly decreased but still some soil-transmitted and food-borne parasitic infections are common in the country [4]. The prevalence of parasitic contamination of vegetables in some provinces of Iran is as follow: Tehran 16.5% [5], Ardabil 25% in vegetables of markets and 29% in vegetables of gardens [6], Shahrood 38% [7], Isfahan 13% [8], Ramhormoz 49% [9], Khoramabad 52.7% [10], and Ahwaz 15.5% [11].

Previous studies have shown that food-borne and soil-transmitted parasitic infections are endemic in Zanjan province. For example, Attayan and colleagues reported that the prevalence of gastrointestinal parasites in kindergartens of Zanjan was 10.25% [12]. In another study that assessed the students of secondary schools, it was found that 1.53% of the children had helminthic infections, and 14.76% had protozoan infection [13]. However, no studies have been conducted on parasitic contamination of vegetables in this region. The present study was designed to detect and evaluate the prevalence of parasitic contamination in vegetables collected from grocery stores of Zanjan in 2014.

2. Material and Methods

This study was conducted for one year (summer 2013 to 2014) in Zanjan city. Totally 352 vegetable samples including leek, parsley, basil, mint, radish, cress and dill were collected from grocery stores by cluster sampling. The samples were collected from 5 different regions (North, South, East, West, and Center) of the city, and 4 stores were selected randomly from each region.

The samples were transferred to the parasitology department, and the edible parts were cut and placed in 10% saline for 5 minutes. The samples were shaken by hand to settle particles and parasitic organisms. Then, the floating vegetables were collected from the surface. The remaining fluid was held still for 24 hours, and afterwards, the supernatants were discarded, and 20 mL of the remaining was filtered through four-layer clean gauze to remove large particles. The filtered fluid was divided into two equal parts and centrifuged for 5 min. at 2000 g. The supernatants were discarded, and the sediment was examined under a light microscope using 10x and 40x objectives. The second part of the samples was examined using saturated common saline solution [14]. Chi-square statistical test was applied to compare the prevalence of parasitic contamination of the samples in different seasons using SPSS 16.0 software and p-value <0.05 was considered as statistically significant.

3. Results and Discussion

Totally 54% of the 352 samples of vegetables were positive for free-living and parasitic organisms, but only 2.8% of the samples had known pathogenic parasites. Observed parasites included *Trichostrongylus* eggs (0.8%), hookworm eggs (0.6%), *Eimeria* oocysts (0.6%), *Sarcocystis* oocysts (0.3%), *Strongyloides* larvae (0.3%), and *Fasciola* eggs (0.3%) (Table 1).

Table1: Frequency table of parasitic contamination of vegetables in grocery stores in Zanjan.

	Frequency(%)
Nonpathogenic Parasite	180 (51.1)
<i>Eimeria</i> oocyst	2 (0.6)
<i>Sarcocystis</i> oocyst	1 (0.3)
Hookworm egg	2 (0.6)
<i>Trichostrongylous</i> egg	3 (0.8)
<i>Strongyloides</i> larva	1 (0.3)
<i>Fasciola</i> egg	1 (0.3)
Negative	162 (46.0)
Total	352 (100)

The seasonal prevalence of parasitic and free-living organisms in contaminated vegetables is presented in Table 2. There was a significant relationship between prevalence of the vegetable

contamination and seasons. The highest prevalence was observed in fall with 90.4% ($p<0.05$).

Table 2: Seasonal prevalences of parasitic contamination of vegetables, Zanjan, Iran.

Season	No	Pathogenic Parasites No (%)	Non-pathogenic	Total No (%)
Spring	81	1 (1.2)	23 (28.4)	24 (29.6)
Summer	83	4 (4.8)	33 (39.8)	37 (44.6)
Fall	94	4 (4.2)	81 (86.2)	85 (90.4)
Winter	94	1 (1.1)	43 (45.8)	44 (46.8)
Total	352	10 (2.8)	180 (51.1)	190 (54.0)

Table 3 shows the prevalences of the parasitic contamination of vegetables according to the different regions of the city. There was no

significant relationship between the prevalence of the parasitic contamination and different regions.

Table 3: Distribution of parasitic contamination of vegetables in different regions of Zanjan, Iran.

Study regions	No	Pathogenic Parasites No (%)	Non-pathogenic Parasite No (%)	Total No (%)
North	72	1 (1.4)	36 (50.0)	37 (51.4)
South	70	2 (2.8)	34 (48.6)	36 (51.4)
East	70	2 (2.8)	37 (52.8)	39 (55.7)
West	70	3 (4.3)	35 (50.0)	38 (54.3)
Center	70	2 (2.8)	38 (54.3)	40 (57.1)
Total	352	10 (2.8)	180 (51.1)	190 (53.9)

Parasites such as *Trichostrongylous*, *Strongyloides stercolaris*, hookworms, and animal and human ascarids spend part of their life cycle in the soil and protozoa such as *Giardia*, *Entamoeba histolytica*, and *coccidia* can contaminate soil and water. Therefore, people who are living in such contaminated places can be easily infected. The contamination rate of vegetables by *Giardia* cysts has been reported in many studies in different

parts of Iran and other countries such as 10.6% in Isfahan [8], 7% in Ardabil [6], 1.8% in Qazvin [15], 34.78% in Shahrood [7], 3.1% in Zabol [16], 6.3% in Ramhormoz [9], 8.2% in Shahr-e-kord [17], 8.1% in Zahedan [18], 22.5% in Amol [19], 13.3% in Ahwaz [11], 6.9% in Khoram abad [10], 6.8% in Golestan [20], 3.1% in Saudia Arabia [21], 10% in Libya [1], and 8.8% in Egypt [22], but in the present study, *Giardia* cysts were not

found in the samples. The environment can be contaminated with *Giardia* cysts through feces. Vegetables become infected with contaminated soil or water. Based on these facts, we probably did not find *Giardia* cysts in the samples because the vegetables in the city are irrigated by sources other than sewage water.

In Amol, contamination frequency of *coccidia* oocysts was reported to be 5.3%. In the present study the contamination frequency of *Eimeria* and *Sarcocystis* oocyst were 0.6% and 0.3%, respectively. *Coccidia* such as *Eimeria* and *Sarcocystis* can infect a large spectrum of hosts. Since other parasites with human origin were not found in this study, it seems that oocyst contamination was due to animal sources. As there are several different species of these parasites, it is not possible to determine the species using direct microscopy or concentration methods, and, therefore, it is not possible to determine the source of contamination.

In the present study, the most frequent contamination was with *Trichostrongylus* eggs.

This result is in line with a study conducted in Tehran in 2002 which reported a 15% prevalence of *Trichostrongylus* contamination [5]. Contamination of vegetables by *Trichostrongylus* in different regions were reported as follows: 15.1% in Isfahan [8], 1.8% in Ramhormoz [9], 0.4% in Yazd [23], 4.3% in Shahr-e-kord [17], 1% in Zahedan [18], 2.8% in Qazvin [15], 6.4% in Amol [19], 3.1% in Zabol [16], 2.9% in Kerman [24], 18% in Korea [25], and 8.9% in Pakistan [26]. *Trichostrongylus* is a zoonotic helminth. In cases that the load of infection in humans is high, it can result in hypersensitivity and other intestinal complications. The presence of the eggs in vegetables, represents using animal manure and/or entrance of ruminants to the farms. To prevent the spread of zoonotic diseases which can be caused by the presence of ruminants on the farms we recommend using walls or hedges around the farms.

Among the cestodes, *Taenia saginata*'s egg is excreted from human feces, but contamination of vegetables with this egg is not the infective source for humans. On the contrary, the eggs of *Echinococcus* species, which are not distinguishable from other taenid eggs and is excreted in dog feces, can infect humans. Contamination rate of vegetables with *taenia* eggs in different regions is as follows: Ardabil 1.4% [6], Isfahan 7.5% (8), Shahrood 6.8% [7], Shahr-e-kord 9.2% [17], Kerman 3.7% [24], Zahedan 13.1% [18], Qazvin 1.8% [15], Amol 3.2% [19], Zabol 23.4% [16], and 22% in Libya [1]. As Shahnazi and Jafari-Sabet mentioned in their study, contamination of water, soil, and vegetables is an important epidemiological factor in the infection of humans [15].

No *Taenia* eggs were observed in this study. In spite of some reports on human hydatid cyst in Zanzan [27], it seems human infection with *Echinococcus granulosus* via infected vegetables is not an important cause of infection in this area.

High prevalence of vegetable contamination with rhabditoid larvae indicates the epidemiological importance of vegetables in the incidence of some parasitic diseases such as *Strongyloides stercoralis* which enters the body through dermal contact. In the present research, only one rhabditoid *S. Stercoralis* larva was diagnosed, based on the small buccal cavity and large genital primordium.

There are few reports of vegetable contamination with *S. Stercoralis* in other studies in Iran because rhabditoid larva of *Strongyloides* is highly similar to that of other free-living larvae thus unrecognizable. In a study conducted by Ezatpour et al, in Khoramabad in 2011, the frequency of *S. stercoralis* infection was determined to be 1.1% and 1.8% in winter and spring, respectively [10]. Al-Binali and colleagues reported the contamination rate of 6.3% from south western Saudi Arabia which is slightly higher than vegetable contaminations with *Strongyloides* in Iran [21]. Zanzan province has a highland climate characterized by cold snowy weather in the mountains and moderate climate in

the plains in Winter time. Zanjan city, the capital of the province, has a semi-dry extremely cold climate, and vegetables are grown locally from late spring to mid-fall, but during colder seasons vegetables are imported from Qazvin and Khuzestan. In 2014, Healthy Vegetable Distribution Program was conducted in Zanjan city through which greenhouse complexes were constructed to reduce the contamination of vegetables [28]. This can account for the low frequency of contamination in the city compared with other regions in the country. In addition, since the samples of the present study were obtained from grocery stores, it is possible to obtain a different result by examining samples directly collected from the farms.

4. Conclusion

It is concluded that the rate of vegetable contamination with parasitic microorganisms in Zanjan city is lower than in other regions of Iran maybe because of not using manure water or using chemical fertilizers instead of human and animal waste fertilizers. However, raw vegetables should be considered as a contaminating factor of parasitic infection and it is still necessary to improve sanitary condition of raw fruits and vegetables.

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