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Parasitic Contamination in Commonly- Consumed Vegetables in Mazandaran Province, Northern Iran

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ABSTRACT

Background: Raw (fresh) vegetables are an important ingredient of healthy diet. Many enteric bacterial, parasitic and viral pathogens could be transmitted by vegetables. Mazandaran province is located in northern Iran with a coastal area and extensive fields for vegetable cultivation. The current study is designed to evaluate the parasitic contamination of fresh vegetables.
Methods: A total of 150 samples of fresh vegetables obtained from markets were examined for parasitic infections using standard methods.
Results: Out of 104 samples (60.3%) were contaminated with parasites. Parsley and radish with prevalence rates of 90% (18/20) and 39.1% (9/23) were the most and least contaminated vegetables. *Free living larva* and *Trichostrongylus* were the most and least common parasites in our results.
Conclusion: It can be concluded that parasitological contamination of raw vegetables may be a health threat to consumers of such products.

1. Introduction

Vegetables are an important part of a healthy human diet because of their nutritional value. Raw vegetables are rich sources of vitamins, dietary fiber and minerals; and their regular feasting is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers [1]. On the other hand, vegetables can be contaminated with enteric bacteria, viral and parasitic pathogens during their cultivation, collection, and transportation processes. The sources of zoonotic

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contamination are usually feces,and faecally - contaminated soil or water [2].

Surface and absorbency of vegetables enable the pathogens to attach and consequently consumption of the raw or slightly cooked vegetables may increase the risk of foodborne infections [3]. In many developing countries, use of insufficiently- treated wastewater to irrigate vegetables has been reported to be responsible for the high rates of contamination with pathogenic and nonpathogenic parasites [4]. The consumption of raw vegetables without washing is an important cause of the transmission of parasitic infection [5].

Several studies have reported the high prevalence of intestinal parasites, mainly in developing countries [6, 2].

The World Health Organization has classified parasites as the sixth among the most harmful causes of human infectious diseases [7]. It is assessed that 60% of the world's population are infected with intestinal parasites (pathogen and nonpathogen), which may be transmitted through direct and indirect contact [8, 9]. Most people are not aware of the parasites and their effects on human health and they do not know where and how parasites are infecting them. Humans may be hosts of over 100 different types of parasites, which include roundworms, tape worms, flukes, and protozoa [10]. Parasites that have been associated with vegetable-borne infections are protozoa cysts, oocysts such as *Entamoeba*, *Giardia*, *Toxoplasma*, helminth *eggs* and larvae such as *Ascaris*, *Fasciola*, *Hymenolepis*, *Taenia*, *Toxocara*, *Trichostrongylus*, *Strongyloides* and Hookworms [11-15]. In developing countries, because of insufficient instruments for routine diagnosis and monitoring of the food-borne pathogens, most infections caused by contaminated vegetables go undetected and the prevalence of contaminations is underestimated [16].

Intestinal parasites such as *Giardia lamblia*, *Blastocystis hominis*, *Strongyloides stercoralis* and *Enterobius vermicularis* have high prevalence rate among the inhabitants of Mazandaran province, Iran [17]. According to human prevalence rates of parasitic infections in different parts of the country and the association between vegetables (especially raw ones) and the infections and insufficient previous surveys conducted to evaluate the presence of parasitic contamination in vegetables in Mazandaran province, the present study was conducted to determine the parasitic contamination in some common green vegetables used for raw consumption in this area.

2. Material and Methods

This cross-sectional study was carried out on raw native vegetables consumed in Mazandaran province, Iran, in spring and summer 2012.

Mazandaran province, with an estimated population of 3059083 is located along the Caspian region in northern Iran.

2.1. Sampling

One hundred and fifty fresh vegetable samples in 7 types (as shown below) were randomly collected from 3 districts (east, west and central) in Mazandaran province from March to September 2012. The selected vegetables included dill, coriander, parsley, leek, basil, radish, and spearmint. The vegetable samples were placed in clean plastic bags and directly transferred to laboratory for parasitological examinations.

2.2. Parasitological procedures

Vegetable samples were washed by vigorous shaking with a suitable quantity of normal saline solution. After Washing with saline the samples were transferred into conical tubes, and centrifuged at 2500 rpm for 5 min [18]. Then, the supernatant was cast off carefully, and the sediment was transferred to microscope slides for parasitological examination with a light microscope under ×100 and × 400 magnifications.

Parasites were identified by morphological characteristics. Normal saline and iodine preparations were examined for parasitic protozoa and helminthes in the laboratory of medical parasitology, Mazandaran University of medical sciences.

2.3. Baermann technique

In this procedure, 10 g (or more) of the soil contacting vegetable roots is put upon the filter and the filter is placed on the funnel full of saline or water which has an evacuating valve. After 2 hours the soil larvae and nematodes move toward water and accumulate inside the funnel tube. At last opening the funnel tap leads the nematodes and larvae to the glass bottle [19].

2.4. Statistical analysis

Statistical analyses were carried out using Chi-square test to assess the differences between proportions at a significance level of 0.05 and 95% CI using SPSS 18 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

One hundred four (60.3%) samples were contaminated with parasites. There was no statistically significant association between the species of vegetable examined and the presence of parasitic contamination ($P < 0.05$). According to table 1, seven spices of vegetables (dill, coriander, parsley, leek, basil, radish, and spearmint) were used in our study for parasitological survey, in which Parsley with prevalence rate of 90% (18/20) and Radish with prevalence rate of 39.1% (9/23) were the most and least contaminated vegetables, respectively.

Table 1: Prevalence of parasitic contaminations in vegetables consumed in Mazandaran province, Iran.

Vegetables	Infection with pathogenic and/or nonpathogenic parasites	
	Number	No (%)
Dill	20	17 (85%)
Coriander	21	18 (85.7%)
Parsley	20	18 (90%)
Leek	23	13 (56.5%)
Basil	21	12 (57%)
Radish	23	9 (39.1%)
Spearmint	22	17 (77.2%)

Samples of the current work were collected from three districts (east, west and center) from Mazandaran province (Table 2); that east areas were more polluted than others, but there was no statistically significant association between the districts surveyed and the presence of contamination ($P > 0.05$).

Table 2: Distribution of intestinal parasites in vegetables consumed from different areas in Mazandaran province, Iran.

Contamination	East		Center		West	
	No	%	No	%	No	%
Polluted	37	74	31	62	36	72
Non polluted	13	26	19	38	14	28
Total	50	100	50	100	50	100

Free living larva and Trichostrongylus were the most and least common parasites in our results, respectively (Table 3). Other detected parasites were *Giardia spp* (7.3%), *Blastocystis spp* (8%), *F. hepatica* (9.3%), *E. coli* (5.3%), *D. dendriticum* (7.3%) and *Taenia egg* (2.6%).

Table 3: Prevalence and intensity of parasites contamination in vegetables consumed in Mazandaran province, Iran.

Infection	No	Percent (%)
One parasite	104	69.33
Mix parasite		
Two parasites	29	19.33
Three parasites	2	1.33
Kind of parasite		
Giardia lamblia	11	7.3
Blastocystis hominis	12	8
Entamoeba coli	8	5.3
Fasciola hepatica	14	9.3
Dicrocoelium dendriticum	11	7.3
Taenia egg	4	2.6
Trichostrongylus	3	2
Free living larva	74	49.3

Vegetable consumption is an important way for the transmission of intestinal parasites and has been shown to be an important cause of foodborne outbreaks in developed and developing countries [20]. Mazandaran is a coastal province. It has extensive agriculture fields for cultivations of different kinds of vegetables. Numerous studies have been conducted in Iran and other parts of the world on the detection of parasites in vegetables. All results revealed high prevalence in all examined vegetables and parasites such as *G. lamblia*, *E. coli*, *A. lumbricoides eggs*, and *Taenia* spp. eggs have been reported [2, 10, 21-30].The results of these researches explained that peoples all over the world crave for raw vegetables which are cultivated in the gardens and fertilized with non-treated soil and it seems that using sewage is a chief agent in contamination of vegetables.

The results of our study shows that the prevalence of parasitic contamination of the vegetable samples was 60.3%.These results were in agreement with another study in Kenya which reported the overall parasitic contamination of the vegetables as 75.9% [6]. Parasitic contaminations of the vegetable in another study in Alexandria, Egypt was also 58% [1]. Daryani et al, (2008) reported that 50% (48/96) of market vegetables and 71% (32/45) of garden vegetables were contaminated with different kinds of parasites [2].

Klapec and Borecka (2012) revealed that 34.7% of the vegetables in conventional farms and 18.9% in organic farms were contaminated with parasites. Geographical location, type and number of examined samples, methods for detection of the intestinal parasites, type of water for irrigation, and post-harvesting handling methods can be causes of these differences [1].

The highest rate of contamination in current study was detected in parsley samples (90.0%).

Radish is a root vegetable and with the lowest contamination in our study (39.1%). It could be concluded that the degree of contamination is associated with the shape and surface of vegetables. According to our results, like many other studies, the parasite prevalence in leafy vegetables was considerably higher, compared to root vegetables [18, 27].

Because of the rough surfaces of the green leafy vegetables such as coriander and parsley, parasitic eggs, cysts and oocysts could attach more easily. Alternatively, vegetables with flat surface as leek and basil had the least prevalence because their smooth surfaces diminish the rate of parasitic attachment [28].

In the present study free living larva, a non-pathogenic parasite and *F. hepatica*, a highly dangerous trematode, were most frequently detected. *Fasciola* eggs were detected in 9.3% of the examined samples. In a study in Ardebil, *Fasciola* eggs were observed in 5% of the samples. In Egypt, *Fasciola* eggs were found in 2.4% of the samples [29]. Vegetables contaminated with the eggs of parasitic round worms may be the source of infestation of humans and other hosts, and they may be the cause of infection in holiday-makers visiting these farms. In the current work, eggs of *Trichostrongylus* were distinguished in 2% (3/150) of the examined vegetables. A study in Philippine reported the detection of *Trichostrongylus* in 6.4% of leafy vegetables examined [30]. In another study in Saudi Arabia T*richostrongylus* was reported in 3.1% of the samples [10]. In some previous study in Iran, *Trichostrongylus* eggs were detected in 0 to 1% of the total examined samples [2, 26].

We detected *Taenia spp*. eggs in 2.6% of samples. We suspect that they would be *Echinococcus spp* based on taxonomical characteristics and could not be recognized from *Taenia spp*. *Taenia spp* are transmitted by water contaminated with faeces of dogs and are infective for humans. Therefore, ingestion of the *embryonated Echinococcus spp.* eggs through contaminated vegetables increases the risk of hydatid cyst infection [26]. In Jordan, 55 patients with hydatid disease were interviewed and all patients gave history of contact with dogs since their childhood, and history of lifelong ingestion of raw vegetables [31]. In Turkey, the rate of

contamination with *Taenia* was 2.7% in carrots, cress, and spinach [21]. In another study in Ankara, Turkey *Taenia* spp. eggs were found in 3.45% of 203 unwashed parsley, green onion, cucumber, and carrot samples [32]. A similar study in Iran, reported that 9.2% of the raw vegetable samples were polluted with *Taeniidae* spp. Eggs [26]. In the present study, 7.3% (11/150) of the samples were contaminated with *Dicrocoelium dendriticum* eggs. Another study in Iran, reported prevalence rate of 6% for *Dicrocoelium dendriticum* eggs in vegetables of markets and gardens [2]. Biologically, the highest health risk is for helminth infections compared with other pathogens because helminthes persist for longer periods in the environment and the infective dose is low [33]. The presence of helminth eggs in different vegetables is mainly associated with contamination of soil rather than contamination of irrigation water [34].

In this study the prevalence of *Giardia spp*. cysts was detected 7.3% and parsley was the highest polluted vegetable. Findings of this study are similar to previous researches in Iran. They were 8.2%, 14%, 6.5%, 9% and 4% in Shahrekord, Jiruft, Tehran, Ardabil and Qazvin, respectively [35, 36, 25, 2, 23]. Another study in Norway, reported 2.1% contamination with *Giardia spp*. in fruits and vegetables [37]. In this study, *E. coli* and B. *hominis* were detected in 5.3% and 8% of samples. Daryani et al, (2008) revealed that 10% of imported vegetables and 18% of cultivated vegetables in Ardabil were contaminated with *E. coli* [2]. In a similar study in Saudi Arabia, B. *hominis* was the most common parasite detected and the prevalence rate was 15.8 % [10]. Although this parasite is non pathogen, it is reflected as a health indicator. This parasite is zoonotic; therefore, it seems that those vegetables have been contaminated with human faces. Thus, the occurrence chance of other intestinal pathogenic and nonpathogenic parasites on vegetables is high. These results may suggest precautions for global food safety and highlight the importance of raw vegetables in threatening public health by transmission of intestinal parasites to humans in this province.

4. Conclusion

According to our results, consumed vegetables are polluted with different pathogens. Media programs should raise awareness on the risks of acquiring intestinal parasites through consumption of raw vegetables, and the importance of suitable washing of vegetables before consumption.

Additional studies are required to survey parasitic contamination in green vegetables in different districts of Iran. Also, further studies should be conducted to evaluate the level of contamination of irrigation water and soil in which green vegetables are cultivated. Different ways of disinfection of raw green vegetables should be improved. In the meantime, educational programs should alert producers to improve the cultivation and harvesting of vegetables, and to monitor the quality of the water used for irrigation.

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