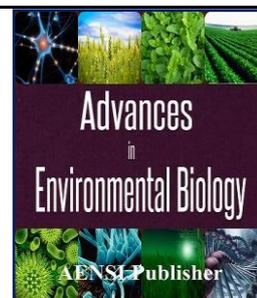




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Assessment of food-borne Parasites in Salad Vegetables in Tabriz city, North-West of Iran

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ABSTRACT

Objective: Fresh vegetables are one of the most important parts of a healthy diet. Raw vegetables are rich sources of vitamins, dietary fiber and minerals; and their regular consumption is associated with reduced risk of cardiovascular diseases, stroke and certain cancers. Raw vegetables can be agent of transmission of intestinal parasites. The main objective of present study was to determine the parasitological contamination of fresh salad vegetables sold in markets of Tabriz city, Iran. **Methods:** A total 120 samples of salad vegetables obtained from Tabriz city markets were examined for helminth eggs and protozoa cysts using standard parasitological methods. **Results:** Helminth eggs and protozoa cysts were observed in 55% (66/120) of examined fresh salad vegetables. From 30 tomato, 30 cucumber, 30 lettuce, and 30 carrot samples, eggs of *Ascaris* spp. were observed in 17%, 73%, 93% and 97%, respectively; eggs of *Toxocara cati* were observed in 10%, 13%, 47% and 43%; and eggs of *Taenia/Echinococcus* were observed in 7%, 26%, 33% and 30%, respectively. Cysts of *Giardia* spp. were detected in 3%, 20%, 7% and 13%, respectively; and *Entamoeba histolytica* cysts were detected only in 7% of tomato samples. **Conclusions:** In conclusion, this study highlighted the importance of raw vegetables as the potential source of transmission of intestinal parasites to humans. Vegetables contaminated with pathogenic parasites, pose health risk to the consumers if consumed without proper cleaning and or cooking.

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INTRODUCTION

An important ingredient of healthy diet is raw (fresh) vegetables. Vegetables can become contaminated with enteric bacterial, viral and parasitic pathogens throughout the process of planting to consumption. The extent of contamination depends on several factors that include, among others, use of untreated wastewater and water supplies contaminated with sewage for irrigation, post-harvest handling, and hygienic conditions of preparation in food service or home settings [1,20]. Fresh vegetables can be agents of transmission of protozoa cysts and helminths eggs and larvae [3,5,8] and outbreaks of intestinal parasitic infections epidemiologically associated with raw vegetables have been reported from developed and developing countries [9,11].

Fresh vegetables are one of the most important parts of a healthy diet. Raw vegetables are rich sources of vitamins, dietary fiber and minerals; and their regular consumption is associated with reduced risk of cardiovascular diseases, stroke and certain cancers. Raw vegetables can be agent of transmission of intestinal parasites [1,2,3]. Incidence of intestinal parasitic infections epidemiologically associated with raw vegetables have been reported in developed and developing countries [4, 5]. Intestinal parasites are widely prevalent in developing countries, probably due to poor sanitation and inadequate personal hygiene [6]. It is estimated that 60% of the world's population is infected with gut parasites (pathogen and non pathogen), which may be transmitted through direct and indirect contact, food, water, soil, vertebrate and arthropod vectors and, rarely from mother to offspring and through contamination of soil with animal wastes and increased application of improperly composted manures to soil in which vegetables are grown also [7, 8]. The consumption of non-properly washed vegetables is a major way for transmission of parasitic contaminations (e.g., *Fasciola*) [9,10]. Several surveys in different parts of the world have shown that vegetables can be agent of transmission of

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protozoa cysts and oocysts (including *Giardia*, *Entamoeba*, *Toxoplasma* and *Isospora*), helminthes eggs and larvae (including *Hymenolepis*, *Taenia*, *Fasciola*, *Whipworm*, *Trichostrongylus* *Strongyloides* and *Hookworms*)[11,12]. The main transmission route for most intestinal parasites is fecal-oral, through contaminated food or water. Protozoan parasites, such as *C.parvum*, *G. lamblia*, *E. histolytica*, and *Cyclospora* sp, and helminthic parasites, including *Ascarislumbricoides*, hookworms (*Necator americanus* and *Ancylostoma duodenale*), and whipworm (*Trichuristrichiura*), are causes of water and foodborne diseases parasites [13,14]. Further tendency in many countries toward eating more raw, or lightly cooked vegetables to preserve taste and heat labile nutrients may also increase the probability of foodborne parasitic infections[14]. On the other hand, in some parts of the world, application of sewage on agricultural land is a customary rule which results in transmission of pathogenic organisms from irrigated soil to crops, grazing animals and humans[15,16]. Protozoa and helminthes are the parasites of primary public health concern for waste water reuse. An important characteristic of these organisms is production of a cyst or ova stage which helps their survival. More than 40 million persons have been infected in the world and more than 10% of the world population are at risk of infection[17]. Wastewater was increasingly used for irrigation in the 1970s and early 1980s. In most developing countries, use of insufficiently treated wastewater to irrigate vegetables has been reported to be responsible for the high rates of contamination with pathogenic parasites[18,19]. The main objective of present study was to determine the parasitological contamination of fresh salad vegetables sold in wholesale and retail markets of Tabriz city.

MATERIALS AND METHODS

In this study a total 120 samples of salad vegetables obtained from retail and wholesale vegetable markets in five area of the Tabriz city (north- west- east- south- city center) of Iran (between 2014 and 2015) were examined. Collected samples of fresh vegetables included 30 lettuce, 30 cucumber, 30 tomatoes and 30 carrots and the each fresh vegetable weighted (100 gr each sample). Samples were put in plastic bags and then transported to the laboratory of Department of Veterinary Parasitology, Tabriz branch, Islamic Azad University for diagnosing helminth-eggs and protozoan cysts. Samples were washed with physiological saline solution (%95 Nacl) in the laboratory and the washing water/saline was left for about 12-24 hours for sedimentation to take place and then top layer water was discarded. 5 ml of the remaining water centrifuged at 3000 (RPM) for 15 minutes. Supernatant was discarded and the residue was carefully collected and examined in logul stain slide for cyst and egg of parasites (three slides for each sample). Next Trichrom stain slids examined for Protozoan were identified (three slides for each sample) under the light microscope by using valid parasitology diagnostic keys[13].

Statistical analysis:

Statistical analyses were performed using SPSS 20.0 (Statistical Package for medical Sciences). All statistical tests were expressed as significant at 95% confidence interval. P-values were calculated using Chi-squares test. A P-value <0.05 was considered statistically significant.

Results:

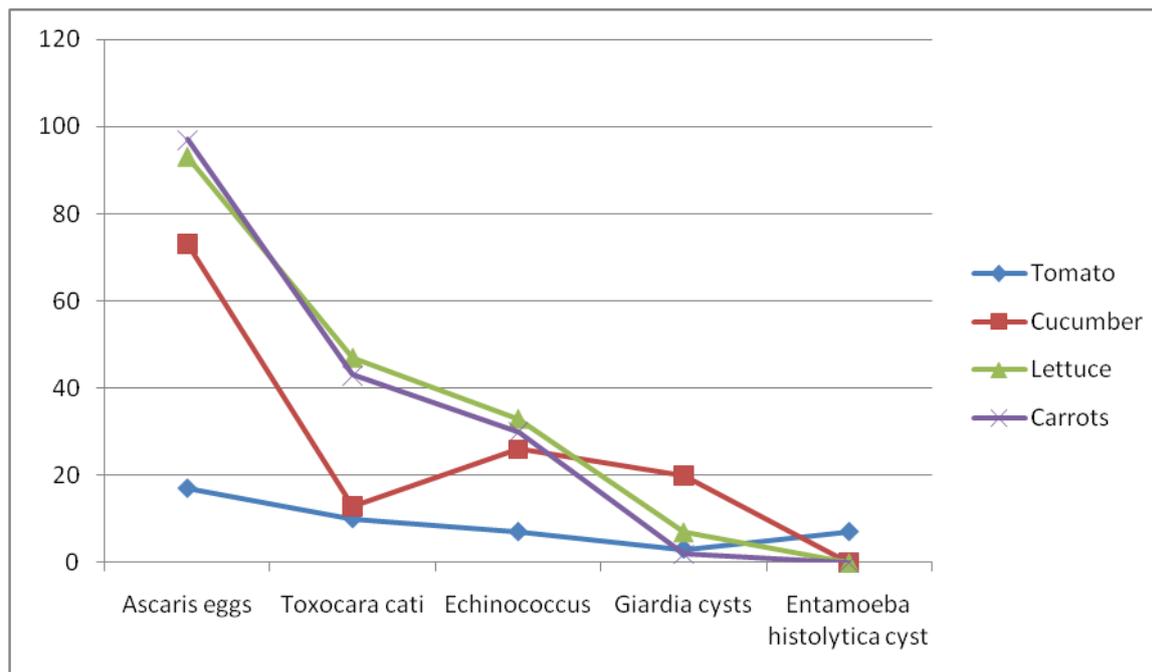
Helminth eggs and protozoa cysts were detected in 55% (66/120) of examined fresh salad vegetables. Samples included 17% (5/30) of tomato, 43% (13/30) of cucumber, 93% (28/30) of carrots and 97% (29/30) of lettuce. Cucumber, carrots and lettuce samples were contaminated significantly more often than those of tomato samples with intestinal parasites. From 30 tomatoes, 30 cucumbers, 30 lettuces, and 30 carrots samples, eggs of *Ascaris* spp. were detected in 17%, 73%, 93% and 97%, respectively; eggs of *Toxocara cati* were observed in 10%, 13%, 47% and 43%; and eggs of *Taenia/ Echinococcus* were seen in 7%, 26%, 33% and 30%, respectively. Cysts of *Giardia* spp. were detected in 3%, 20%, 7% and 13%, respectively; and *Entamoeba histolytica* cysts were detected only in 7% of tomato samples. *Toxocara cati* and eggs of *Taenia/Echinococcus* were detected significantly in total lettuce and carrot samples more than in total tomato and cucumber samples.

Some of non-pathogenic parasites, as free-living larva and mites, were observed in examined samples but we didn't considered them in our results. Also we found *fasciola* eggs in one of our samples. Because of non-pathogenic effects of *fasciola* egg on human compared to metacercarial form of *fasciola*, we did not consider that in results of this study.

Table-1 shows the prevalence of intestinal parasites in fresh salad vegetables from markets in Tabriz city, Iran. Also figure-1 shows the percentage of contaminated samples in each type of salad vegetable in Tabriz city, Iran.

Table 1: Prevalence of pathogenic intestinal parasites in fresh salad vegetables from markets in Tabriz city, Iran.

Total n= 120	Carrots n= 30	Lettuce n= 30	Cucumber n= 30	Tomato n= 30	<i>Parasite</i>
No. (%) positive	No. (%) positive	No. (%) positive	No. (%) positive	No. (%) positive	
84(70)	29(97)	28(93)	22(73)	5(17)	<i>Ascaris eggs</i>
34(28)	13(43)	14(47)	4(13)	3(10)	<i>Toxocara cati</i>
29(24)	9(30)	10(33)	8(26)	2(7)	<i>Taenia/ Echinococcus</i>
13(11)	4(13)	2(7)	6(20)	1(3)	<i>Giardia cysts</i>
2(2)	0	0	0	2(7)	Entamoeba histolytica cyst

**Fig. 1:** The percentage of contaminated samples in each type of salad vegetable in Tabriz city, Iran.**Discussion:**

Consumption of raw vegetables plays an important role in transmission of parasitic contamination [1,2,13]. A total sample of 120 salad vegetables obtained from wholesale and retail markets of Tabriz were examined for determination of helminth eggs and P rotozoan cysts, using standard methods. Helminth eggs and protozoa cysts were detected in 55% (66/120) of examined fresh salad vegetables. Samples included 17% (5/30) of tomato, 43% (13/30) of cucumber, 93% (28/30) of carrots and 97% (29/30) of lettuce collected from markets of Tabriz city, Iran. High prevalence of intestinal parasites such as Giardia, *Ascaris lumbricoides* and *Taenia* spp. was reported among the inhabitants of Ardabil city, Iran [18]. In Alqalamoun region of Syria a total sample of 137 fresh vegetables were randomly collected [17]. All samples were examined according to standard methods for detection of protozoan cyst, oocysts, helminth eggs as well as larva. Intestinal parasites were detected in 43/137 (31.38%) samples, *Giardia lamblia* cysts were the most prevalent parasites (13.13%) followed by *Entamoeba* spp. cysts (8.75%), *Enterobius vermicularis* eggs (5.83%), and *Ascaris lumbricoides* eggs (3.64%). The highest contaminated vegetables were lettuce and parsley (32.56%) and the lowest was radish (4.65%). These findings provide evidence for high risk of acquiring parasitic infection from the consumption of raw vegetables in Alqalamoun region, Syria. Helminth eggs and protozoa cysts were detected in 31.386% (43/137) of examined fresh vegetables. Most parasites detected in the samples were assigned to *Giardia lamblia* (13.13%), *Ascaris lumbricoides* (3.64%). Lettuce and parsley were the most contaminated in contrast. 32.14% of parsley had been contaminated with parasites in an evaluation study of the edible vegetables in Qazvin, Iran [18,19]. In present investigation, *Giardia* spp. cysts were detected in 13.13% of the total sample vegetables. In Egypt, *Giardia* spp. Cysts was found in 6.7% of different vegetables. Reports in Iran were 8.2% in Shahrekord, 14% in Jiruft and 6.5% in Tehran. The second most prevalent parasitic stage was *Entamoeba* spp. cyst 8.75%. Such result was detected in Al-Nassiriyah city, Iraq (25.30%). A high level of contamination of the environment with the eggs

of intestinal parasites such as *Ascaris* spp. observed in many regions of the world which can be associated with high fertility of these parasites (Garcia and Bruckner 1993). Previous study from Saudi Arabia reported the *Giardia* spp. cysts in 31.6% of leafy examined vegetables. *Giardia* has the potential to cause foodborne disease through transfer from water; outbreaks of foodborne disease have occurred but tended to involve fewer reported cases than those attributed to the drinking water supply [9,10]. In another study by Abougrain AK, et al. in Libya, Helminth eggs and *Giardia* cysts were detected in 58% (73/126) of examined fresh salad vegetables. Samples included 14% (5/36) of tomato, 42% (15/36) of cucumber, 96% (26/27) of lettuce and 100% (27/27) of cress. Cucumber, lettuce and cress samples were contaminated significantly more often than those of tomato samples with intestinal parasites [2]. A study from Saudi Arabia reported detection of *Ascaris lumbricoides* in 16% of examined leafy vegetables (reports on the prevalence of *Toxocara* spp., [1]. in fresh vegetables are few in the literature) and it detected *Toxocara* spp. in 1.5% in unwashed raw vegetables used for salad. We detected eggs of *T. cati* and *T. canis* in 26% and 18% of studied fresh salad vegetables, respectively. *Giardia* was the first of these organisms to be widely associated with human disease, and there have been many documented cases of waterborne giardiasis since the 1970s. The source of the eggs of *Toxocara* spp. may be domestic animals (dogs and cats), and wild animals (foxes, wolves) in recent years. Also in our study in Tabriz city of Iran *Entamoeba histolytica* cysts were detected only in 7% of tomato samples that was not reported in other studies of other countries in recent years and this protozoa can induce many pathogenic effects in vegetables consumers. Our report of *Entamoeba histolytica* cysts in tomato is the first report of these protozoa in Tabriz city of Iran in 2015.

Conclusion:

In conclusion, this study highlighted the importance of raw vegetables as the potential source of transmission of intestinal parasites to humans. Vegetables contaminated with pathogenic parasites, pose health risk to the consumers if consumed without proper cleaning and or cooking. Prevention of contamination remains the most effective way of reducing foodborne parasitic infection. Consumers should always observe the basic principle of food and personal hygiene thorough washing vegetables before eating and washing hands before meal. Parasitological contamination of raw salad vegetables sold in markets in Tabriz city, Iran may pose a health risk to consumers of such products.

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Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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