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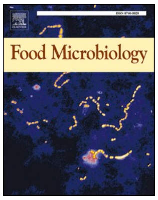

Zoonotic parasitic organisms on vegetables: Impact of production system characteristics on presence, prevalence on vegetables in northwestern Iran and washing methods for removal

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A R T I C L E I N F O

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A B S T R A C T

Fresh vegetables are essential components of a healthy and nutritious diet, but if consumed raw without proper washing and/or disinfection, can be important agents of transmission of enteric pathogens. This study aimed to determine the prevalence of zoonotic parasites on vegetables freshly harvested and "ready to eat" vegetables from greengrocers and markets in northwestern Iran. In addition, the effect of cropping system and season on contamination levels were assessed as well as the efficacy of washing procedures to remove parasites from the vegetables. A total of 2757 samples composed of field ($n = 1, 600$) and "ready to eat" ($n = 1157$) vegetables were analyzed. Vegetables included leek, parsley, basil, coriander, savory, mint, lettuce, cabbage, radish, dill, spinach, mushroom, carrot, tomato, cucumber and pumpkin. Normal physiological saline washings from 200 g samples were processed using standard parasitological techniques and examined microscopically. A total of 53.14% of vegetable samples obtained from different fields and 18.23% of "ready to eat" vegetables purchased from greengrocers and markets were contaminated with different parasitic organisms including; *Entamoeba coli* cysts, *Giardia intestinalis* cysts, *Cryptosporidium parvum* oocysts, *Fasciola hepatica* eggs, *Dicrocoelium dendriticum* eggs, *Taenia* spp. eggs, *Hymenolepis nana* eggs, *Ancylostoma* spp. eggs, *Toxocara cati* eggs, *Toxocara canis* eggs, *Strongyloides stercoralis* larvae, and *Ascaris lumbricoides* eggs. In both field and "ready to eat" vegetables, the highest parasitic contamination was observed in lettuce with a rate of 91.1% and 55.44%, respectively. The most common parasitic organism was *Fasciola hepatica*. A seasonal difference in contamination with parasitic organisms was found for field and "ready to eat" vegetables ($P < 0.05$). There was a significant difference in the recovery of parasitic organisms depending on the washing method with water and dishwashing liquid being the least effective. Proper washing of vegetables is imperative for a healthy diet as the results of this study showed the presence of zoonotic parasites from field and ready to eat vegetables in Iran.

1. Introduction

Vegetables are an essential part of a healthy human diet owing to their nutritional value including serving as an important source of vitamins (B-complex, C, A, and K), minerals (calcium, magnesium, potassium, iron, beta-carotene) and dietary fiber. Their regular consumption is associated with a reduced risk of chronic diseases such as hypertension, diabetes, atherosclerosis and cancer ((López-Gálvez et al., 2010). However, vegetables can be contaminated with enteric bacteria, viruses and zoonotic parasites. Several surveys in different parts of the world have shown that vegetables can be agents for transmission of protozoan cysts and oocysts (e.g., *Entamoeba*, *Cyclospora*, *Toxoplasma* and *Isospora*) and helminth eggs and larvae (e.g., *Hymenolepsis*, *Taenia*, *Fasciola*, *Toxocara*, *Ascaris*, *Trichostrongylus* and hookworms) (Dvuong et al., 2006) with the consumption of raw vegetables playing a major epidemiological role in the transmission of food-borne parasitic zoonoses such as Giardiasis, Strongyloidiasis, Fascioliasis, Fasciolopsiasis and Echinococcosis (Abougrain et al., 2010; Adamu et al., 2012; Adanir and

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Tasci, 2013; Adenusi et al., 2015; De La Vega-Miranda et al., 2012; Duedu et al., 2014; El Said Said, 2012; Eraky et al., 2014; Erdoğrul and Şener, 2005; Kozan et al., 2005; Maikai et al., 2012b; Monge and Arias, 1996; 2012; 2012; Omowaye and Toluhi 2011; Samaliev and Mohamedova, 2011; Santos et al., 2012). Intestinal parasite infection as a cause of malnutrition/growth stunting is well documented and is caused by a decline in food intake and/or an increase in nutrient wastage (Stephenson, 1994). Moreover, many studies indicate that parasitic infections cause malabsorption, diarrhea, and other states of poor health.

The contamination of vegetables with parasitic organisms can occur throughout the process from planting to consumption (Abougrain et al., 2010). Sources of contamination of raw vegetables during planting and growing include the use of raw manure as fertilizer, the use of untreated wastewater for irrigation and inadvertent exposure to animal or human feces (Khan et al., 2017). Raw manure can contain large numbers of pathogenic microorganisms, which potentially pose risks to human health. Concentrations of some pathogens exist at levels of millions to billions per gram of wet weight feces or millions per milliliter of urine (Kumar et al., 2013). Increasing water scarcity in some climatic regions with agriculture-based economies forces people to use untreated wastewater for irrigation of crops (Fattal et al., 1986). Latrines located near vegetable fields and gardens and access of animals to vegetable fields and gardens could lead to inadvertent fecal contamination. Post-harvesting, poor hygienic procedures in food services can result in contamination and the tendency of people to eat raw or slightly cooked vegetables to protect heat-labile nutrients may increase the risk of food-borne, and specifically, parasitic, infections.

A proper and standardized sanitizing process is essential for the safe consumption of vegetables and for decreasing the risk of food-borne illness to the consumer. Washing and sanitizing are the primary measures used to reduce the risk of consuming contaminated vegetables. Chlorine, in the form of sodium hypochlorite, is the active ingredient commonly used for sanitizing leafy vegetables (Fukumoto et al., 2002). Another widely used sanitizing agent is acetic acid, which often is used in the form of vinegar in various dilutions (Neto et al., 2012).

The aim of this study was to determine the overall and seasonal prevalence of potentially pathogenic parasites on vegetables harvested from production systems with different characteristics (fencing, irrigation, sanitary toilet, fertilizer type) and “ready to eat” vegetables from greengrocers and markets in Tabriz, northwestern Iran. While several studies have reported the prevalence of parasite contamination of vegetables in Iran and elsewhere, few have assessed seasonal differences and production system characteristics contributing to contamination (Daryani et al., 2008; Ezatpour et al., 2013; Fallah et al., 2012; Garedaghi et al., 2011; Gharavi et al., 2002; Saki et al., 2013; Siyadatpanah et al., 2013; Yakhchali and Ahmadiashtiani, 2004). Moreover, we investigated the various washing procedures on the ability to remove parasites from different vegetables.

2. Materials and methods

2.1. Study area

This study was carried out in Tabriz (38° 4′N and 46° 18′E), one of the largest cities of Iran with a human population of over 1.5 million. The climate is cold semi-arid with regular seasons and mean daily temperatures ranging from slightly below 0 °C–26 °C. The annual rainfall is approximately 320 mm. With the exception of mushrooms, vegetable production is outdoors in fields ranging from 10 to 20 ha with production in winter continuing with the use of nylon row tarps. Mushrooms are grown in greenhouses. Wastewater, untreated raw sewage obtained from canals leading from city discharge points to the sewage treatment plant, is used to irrigate crops and fertilizers (animal and/or chemical) are used on more than 80% of the fields and in greenhouses. Some production areas are fenced and some have sanitary toilets.

2.2. Sample collection

A total of 2757 samples of commonly consumed vegetables were collected from fields under different crop production systems and greengrocers and markets in the rural and urban areas of Tabriz between April 2018 and March 2019. Of these samples, 1600 were taken directly from fields (or greenhouses in the case of mushrooms) with sampling during the spring (April, May and June; 400), summer (July, August and September; 475), autumn (October, November and December; 400) and winter (January, February and March; 325). Vegetable fields sampled were primarily in urban areas with some in peri-urban areas. “Ready to eat” vegetables (1157 samples), which have typically been washed using traditional methods which include soaking in water for <10 min and then rinsing, were acquired from greengrocers and markets with samples also collected in the spring (317), summer (419), autumn (221) and winter (200). Fields and greenhouses (n = 32) for sampling were selected to represent sixteen crop production systems used in the region with 50 samples from each location (Table 1), while the greengrocers and markets represented 95% of the vegetable marketing activities in the region. Sixteen commonly consumed vegetables were examined from the fields and greenhouses and 12 “ready to eat” vegetables from the greengrocers and markets: leek (*Allium porrum*), parsley (*Petroselinum crispum)*, basil (*Ocimum basilicum*), radish (*Raphanus sativus),* coriander (*Coriandrum sativum*), dill (*Anethum graveolens*), mint (*Mentha piperita)*, savory (*Satureja hortensis*), cabbage (*Brassica oleracea var capitate*; fields only), spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), mushroom (Agaricus bisporus; greenhouses only), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*; fields only) and pumpkin (*Cucurbita maxima*; fields only). Samples were randomly selected at each location using a transect of the field/greenhouse and collecting from five locations, with each sample (0.3–0.5 kg) collected separately in a clean nylon bag. Samples were then transferred to the parasitology research laboratory of Tabriz University, Iran.

2.3. Assessment of washing methods

To assess different washing procedures on the ability to remove parasites from vegetables, an additional 1600 samples were collected from A-P fields (Table 1), with equal numbers from each production system and equal numbers of each vegetable type allocated to each washing method. Four washing methods were used with 400 samples analyzed using each method. The methods were:

a. Potable water with dishwashing liquid (traditionally washed): the vegetables were immersed in potable tap water inside a sink with two drops of dishwashing liquid. The amount of water was approximately 3–5 L per 1 kg of vegetables. Vegetables were left in the water for approximately 6–7 min for sedimentation of mud and dust. After soaking, the vegetables were collected and placed in a wood or plastic basket and rinsed for 1.5–2 min with potable tap water.
b. 1% vinegar/potable water solution: the vegetables were processed as described for potable water and dishwashing liquid.
c. 1% lemon juice/potable water solution: the vegetables were processed as described for potable water and dishwashing liquid.
d. 0.95% $Ca(ClO)_2$/potable water solution: the vegetables were washed with potable tap water to remove mud and dust, disinfected by immersion for 30 min in a solution containing 200 ppm calcium hypochlorite (based on dilution of concentrated $Ca(ClO)_2$), and finally rinsed in an automated fruit-vegetable washer (EASTECH, model SXQ8-BA, Guangdong, China) for 10 min (Fallah et al., 2012).

After washing, vegetables were examined for remaining protozoan cysts and helminth eggs as described in section 2.4 with the number of vegetables still containing parasitic organisms recorded.

Table 1
Types of crop production systems from which samples were harvested.

Field code	Fence		Toilet		Irrigation		Fertilizer	
	Yes	No	Yes	No	River water	Wastewater	Chemical	Animal
A	X		X		X		X	
B	X		X		X			X
C	X		X			X	X	
D	X		X			X		X
E	X			X	X		X	
F	X			X	X			X
G	X			X		X	X	
H	X			X		X		X
I		X	X		X		X	
J		X	X		X			X
K		X	X			X	X	
L		X	X			X		X
M		X		X	X		X	
N		X		X	X			X
O		X		X		X	X	
P		X		X		X		X

Note: two of each type of production system were included in the study for a total of 32 fields.

2.4. Parasite recovery from samples

A weighed sample (200 g) was washed by vigorous shaking with 1 L of physiological saline solution (0.95% NaCl). The vegetable samples were removed and the remaining wash water was left to sediment for approximately 12 h (Tello et al., 2012). The supernatant water was discarded and the sediment was passed through sieve (425 μm), in order to eliminate large particles and detect larvae and free-living nematodes. The final 50 mL volume was then centrifuged at 2000 g for 5 min. Finally, the supernatant was discarded and three direct smears of the sediment were examined under a light microscope using 40× and 100× objectives. The remaining sediment was resuspended in zinc sulphate flotation solution (spg 1.18) and centrifuged at 1000 g for 3 min. After centrifugation, a convex meniscus on the top of the tube was created by adding flotation solution and covered by a clean coverslip. After 30 min, the coverslip was removed and examined under a light microscope using ×40 and ×100 objectives. The various eggs of the parasites were identified based on morphological details as described by Soulsby (1982). Modified Ziehl-Neelsen stained smears were prepared for detection of coccidian protozoal oocysts including *Cryptosporidium* spp., *Isospora* spp., and *Cyclospora cayetanensis* (Henriksen and Pohlenz, 1981).

2.5. Data analysis

All data were tabulated and the percent of each vegetable type contaminated with parasite cysts or eggs and the variety of parasites recovered determined. Pearson's chi-square test was used to compare the frequency of contamination with parasites between all 16 vegetables directly harvested from fields (greenhouses in the case of mushrooms) and between all of market "ready to eat" vegetables assessed. In the case of statistical significance, residual analysis was used to determine which vegetables were most likely influencing the result; vegetables with a residual of 3 or greater were considered to most likely be influencing the significant result in the chi-square. To determine if the number of parasite species seen differed among directly harvested vegetables and among "ready to eat" vegetables, a regression analysis was used; individual vegetable to vegetable comparisons were not made. The chi-square test also was used to analyze frequency differences by season and by washing method. The differences were considered statistically significant at $P < 0.05$. A best subsets regression analysis was performed to determine which cropping system characteristics could be used to determine the percent of vegetables contaminated. Specifically, the predictors presence or absence of fencing, type of fertilizer, type of irrigation and presence or absence of a toilet were used to determine which singly or in combination would most likely predict the outcome defined as the percent of contaminated vegetables using adjusted R^2. In addition, exploratory analysis, including a best subsets regression, was performed to determine if the presence of parasitic organisms known to come only from cats and dogs could be predicted by cropping system characteristics. Statistics were performed using STATA® 16 (Stata Corp LLC, College Station, Texas, USA) and Minitab®18 (Minitab LLC, State College, Pennsylvania, USA).

3. Results

3.1. Distribution of parasite species identified on different vegetable samples

A total of 53.14% (845/1600) of vegetable samples obtained from different production systems and 18.23% (211/1157) of "ready to eat" vegetables purchased from greengrocers and markets were contaminated with different parasitic organisms (Tables 2 and 3). The parasites detected in commonly consumed vegetable samples were *Entamoeba coli* cysts, *Giardia intestinalis* cysts, *Cryptosporidium parvum* oocysts, *Fasciola hepatica* eggs, *Dicrocoelium dendriticum* eggs, *Taenia* spp. eggs, *Hymenolepis nana* eggs, *Ancylostoma* spp. eggs, *Toxocara cati* eggs, *Toxocara canis* eggs, *Strongyloides stercoralis* larvae, and *Ascaris lumbricoides* eggs. Based on the chi-square analysis comparing all harvested vegetables together, the percent contaminated with parasites was significantly different with lettuce and leek having residuals >3. Lettuce had the highest (91.1%) parasitic contamination rate followed by leek (90%) and cabbage (67.27%) and the lowest parasitic organism contamination was on coriander (38.2%), cucumber (38.09%), tomato (37.63%) and savory (30.12%). The variety of parasites seen also were statistically significantly different; the highest variety of parasites were found on leek and lettuce (mean 3.5: range 0–9) with savory having the lowest variety of parasites (mean 0.3; range 0–1). The most frequently recovered parasitic organism was *Fasciola hepatica* (20.31%).

Of the twelve types of "ready to eat" vegetable samples, the highest number of contaminated samples was detected in lettuce (55.44%) while the least number of contaminated samples was detected in savory (2.50%). Based on the chi-square analysis, the percent contaminated with parasites was significantly different among the vegetables; the vegetables most likely influencing this result with the highest residuals were lettuce, leek, savory, mint and dill. The variety of parasites seen also were statistically different with the widest variety of parasites found on leek with the least on savory. As with vegetables directly harvested, the most common parasitic organism was *Fasciola hepatica* (6.22%); however, unlike the harvested vegetables, neither *Dicrocoelium dendriticum* eggs nor *Ancylostoma* spp. eggs were recovered from market

Table 2
Parasites with zoonotic potential identified on vegetables in Tabriz, Iran.

Vegetable	Number sampled	Number (percent) of sampled vegetables containing each parasite species											
		E. c	G. i	C. p	F. h	D. d	T. s	H. n	A. s	T. ct	T. cn	S. s	A. l
Vegetables at time of harvest direct from the field													
Leek	100	14 (14.0)	23 (23.0)	24 (24.0)	67 (67.0)	51 (51.0)	20 (20.0)	15 (15.0)	23 (23.0)	47 (47.0)	39 (39.0)	0	25 (25.0)
Parsley	95	23 (24.2)	16 (16.8)	24 (25.3)	8 (8.4)	0	14 (14.7)	8 (8.4)	0	19 (20.0)	19 (20.0)	0	20 (21.1)
Basil	90	13 (14.4)	12 (13.3)	0	13 (14.4)	0	17 (18.9)	2 (2.2)	5 (5.6)	5 (5.6)	10 (11.1)	0	35 (38.9)
Coriander	110	14 (12.7)	13 (11.8)	0	3 (2.7)	0	15 (13.6)	1 (0.9)	0	9 (8.2)	2 (1.8)	10 (9.1)	0
Savory	83	9 (10.8)	0	0	2 (2.4)	0	9 (10.8)	1 (1.2)	0	4 (4.2)	0	0	0
Mint	106	13 (12.3)	5 (4.7)	0	10 (9.4)	15 (14.2)	5 (4.7)	11 (10.4)	2 (1.9)	17 (16.0)	9 (8.5)	0	0
Lettuce	112	6 (5.4)	39 (34.8)	51 (45.5)	30 (26.8)	44 (39.3)	31 (27.7)	19 (11.0)	25 (22.3)	45 (40.2)	37 (33.0)	2 (1.8)	48 (43.6)
Cabbage	110	6 (5.5)	3 (2.7)	10 (9.1)	21 (19.1)	29 (26.4)	0	12 (10.9)	14 (12.7)	11 (10.0)	7 (6.4)	31 (28.2)	0
Radish	99	0	10 (10.1)	0	7 (7.1)	23 (23.2)	12 (12.1)	0	0	6 (6.1)	0	0	0
Dill	97	18 (18.6)	0	0	0	13 (13.4)	2 (2.1)	0	0	1 (1.0)	5 (5.2)	0	0
Spinach	93	0	6 (6.6)	0	22 (23.7)	10 (10.8)	1 (1.1)	0	0	5 (5.3)	10 (10.8)	0	0
Mushroom	102	2 (2.0)	0	1 (1.0)	30 (29.4)	14 (13.7)	0	2 (2.0)	0	20 (19.6)	12 (11.8)	5 (4.9)	0
Carrot	105	10 (9.5)	8 (7.6)	3 (2.9)	29 (27.6)	22 (21.0)	6 (5.7)	0	0	25 (23.8)	20 (19.0)	18 (17.1)	8 (7.6)
Tomato	93	9 (9.7)	5 (5.4)	0	24 (25.8)	18 (19.4)	4 (4.3)	0	0	23 (24.7)	16 (17.2)	0	0
Cucumber	105	10 (9.5)	6 (5.7)	0	34 (32.4)	27 (25.7)	0	9 (8.6)	0	23 (21.9)	26 (24.8)	17 (16.2)	3 (2.9)
Pumpkin	100	13 (13.0)	0	0	23 (23.0)	17 (17.0)	11 (11.0)	0	0	16 (16.0)	9 (9.0)	14 (14.0)	0
Total	1600	160 (10.1)	146 (9.2)	113 (7.1)	323 (20.3)	283 (17.8)	147 (9.2)	80 (5.0)	69 (4.3)	276 (17.4)	221 (13.9)	97 (6.1)	139 (8.7)
"Ready to eat" vegetables from markets and restaurants													
Leek	98	6 (6.1)	0	12 (12.2)	42 (42.9)	0	25 (25.5)	3 (3.1)	0	21 (21.4)	4 (4.1)	2 (2.0)	3 (3.1)
Parsley	103	0	0	1 (1.0)	0	0	3 (2.9)	0	0	0	0	5 (4.9)	2 (1.9)
Basil	120	0	0	0	3 (2.5)	0	5 (4.2)	0	0	10 (8.3)	1 (0.8)	4 (3.3)	0
Coriander	74	0	0	0	0	0	1 (1.3)	0	0	0	0	3 (4.1)	2 (2.7)
Savory	80	0	0	0	0	0	2 (2.5)	0	0	0	2 (2.5)	0	0
Mint	110	0	0	3 (2.7)	0	0	0	1 (0.9)	0	1 (0.9)	0	2 (1.8)	0
Lettuce	101	8 (7.9)	3 (3.0)	3 (3.0)	0	0	19 (18.8)	6 (5.9)	0	15 (14.9)	9 (8.9)	3 (3.0)	12 (11.9)
Radish	100	0	0	14 (14.0)	0	0	1 (1.0)	0	0	0	1 (1.0)	0	6 (6.0)
Dill	100	0	0	0	3 (3.0)	0	3 (3.0)	0	0	0	0	1 (1.0)	4 (4.0)
Spinach	88	0	3 (3.4)	14 (15.9)	0	0	6 (6.8)	1 (1.1)	0	0	3 (3.4)	2 (2.3)	5 (5.9)
Carrot	103	0	6 (5.8)	0	20 (19.4)	0	2 (1.9)	0	0	0	0	0	8 (7.8)
Cucumber	80	0	15 (18.8)	1 (1.3)	4 (5.0)	0	0	0	0	5 (6.3)	3 (3.8)	1 (1.3)	3 (3.8)
Total	1157	14 (1.2)	27 (2.3)	48 (4.1)	72 (6.2)	0	67 (5.8)	11 (1.0)	0	52 (4.5)	23 (2.0)	23 (2.0)	44 (3.8)

Abbreviations: E. c: Entamoeba coli cyst, G. i: Giardia intestinalis cyst, C. p: Cryptosporidium parvum oocyst, F. h: Fasciola hepatica egg, D. d: Dicrocoelium dendriticum egg, T. s: Taenia spp. egg, H. n: Hymenolepis nana egg, A. s: Ancylostoma spp. egg, T. ct: Toxocara cati egg, T. cn: Toxocara canis egg, S. s: Strongyloides stercoralis larva, A. l: Ascaris lumbricoides egg.

vegetables.

3.2. Distribution of parasites by crop system

Table 4 shows a comparison of the parasitic contamination of 1600 vegetable samples collected from 16 vegetable cropping systems (2 of each type), based on the presence/absence of a fence, presence/absence of a sanitary toilet, type of irrigation (untreated wastewater and river water) and fertilizer (animal and chemical). Cropping systems that used wastewater and animal fertilizer had the highest number of vegetables with parasites (96.7 and 93.3%, respectively) and contamination levels, based on the chi-square, were statistically different between river water vs wastewater and between chemical fertilizer and animal fertilizer. Lack of a fence also resulted in a higher number of contaminated vegetables (75.6%) compared to having a fence and the difference was statistically significant. Presence or absence of a sanitary toilet resulted in more similar contamination levels (59.8 vs 46.2%) and, based on the chi-square, these differences were not statistically different.

While the cropping system characteristics fence vs no fence, river water vs wastewater and animal vs chemical fertilizer resulted in statistically significant differences in percent of vegetables contaminated, none of these factors were able to predict vegetable contamination on their own based on the best subsets regression analysis. In the regression model, all four cropping system characteristics are required to achieve a

Table 3
Vegetables with parasites with zoonotic potential in Tabriz, Iran: number with parasites and diversity of parasite species.

Vegetable	Vegetables at time of harvest direct from the field			"Ready to eat" vegetables from markets and restaurants		
	Number sampled	Number (percentage) of samples containing parasites	Mean number of parasite species (min; max)	Number sampled	Number (percentage) of samples containing parasites	Mean number of parasite species (min; max)
Leek	100	90 (90.0)	3.5 (0; 9)	98	45 (45.9)	1.20 (0; 8)
Parsley	95	40 (42.1)	1.6 (0; 8)	103	8 (7.8)	0.11 (0; 2)
Basil	90	35 (38.9)	1.2 (0; 6)	120	11 (9.2)	0.19 (0; 4)
Coriander	110	42 (38.2)	0.6 (0; 4)	74	3 (4.1)	0.08 (0; 3)
Savory	83	25 (30.1)	0.3 (0; 1)	80	2 (2.5)	0.05 (0; 2)
Mint	106	68 (64.2)	0.8 (0; 4)	110	5 (4.5)	0.06 (0; 2)
Lettuce	112	102 (91.1)	3.5 (0; 9)	101	56 (55.4)	0.76 (0; 6)
Cabbage	110	74 (67.3)	1.3 (0; 7)	NA	NA	NA
Radish	99	58 (58.6)	0.6 (0; 1)	100	14 (14.0)	0.22 (0; 4)
Dill	97	39 (40.2)	0.4 (0; 1)	100	5 (5.0)	0.11 (0; 3)
Spinach	93	54 (65.1)	0.6 (0; 1)	88	20 (22.7)	0.39 (0; 6)
Mushroom	102	50 (49.0)	0.8 (0; 7)	NA	NA	NA
Carrot	105	42 (40.0)	1.4 (0; 9)	103	22 (21.4)	0.35 (0; 3)
Tomato	93	35 (37.6)	1.1 (0; 6)	NA	NA	NA
Cucumber	105	40 (38.1)	1.5 (0; 7)	80	20 (25.0)	0.40 (0; 5)
Pumpkin	100	51 (51.0)	1.0 (0; 5)	NA	NA	NA
Total	1600	845 (52.8)	1.3 (0; 9)	1157	211 (18.2)	0.33 (0; 8)

Based on a chi-square analysis, at $p < 0.05$, the frequency of parasite contamination was significantly different among the vegetables within each vegetable group, harvested and "ready to eat".
Based on a regression analysis, at $p < 0.05$, the number of parasite species differed significantly among the vegetables within each vegetable group, harvested and "ready to eat". NA: Not available.

R^2 of 86.2 with models containing three of the characteristics achieving a R^2 of 76.4 or less. However, individual cropping system characteristics might be effective in modelling the percent of vegetables likely to be contaminated with specific types of parasites. An exploratory best subsets analysis was performed considering only *T. canis* and *T. cati* eggs, two parasites only from dogs and cats. In this case, a regression model with only presence or absence of a fence resulted in a R^2 of 85, while inclusion of all four crop system characteristics resulted in a R^2 of 86.

3.3. Percentage frequency of occurrence of parasites in different seasons

The percent of vegetables contaminated by season are presented in Table 5. For both groups of vegetables, directly harvested and "ready to eat", there was a season effect in regards to percent contaminated with parasitic organisms ($P < 0.05$). The highest rate of parasitic contamination in vegetable samples directly harvested in different seasons was found in summer (75.15%) and the lowest in winter (26.15), with these two seasons having residuals >3 in the chi-square analysis. For "ready to eat" vegetables from markets, the highest rate of parasitic contamination in samples was found in spring (34.38%) while the lowest contamination was in winter (10.50%); based on the chi-square residuals, the statistically significant season difference is most likely attributed to the spring, the only season with a residual >3.

3.4. The comparison of washing procedures on parasitic contamination of vegetables

The washing methods were significantly different in their ability to remove parasitic organisms based on the chi-square. Washing with water and dishwashing liquid removed the least number of parasites, with 160 of 400 vegetables (40%) still being contaminated (Table 6). In contrast, 0.95% Ca removed the most parasitic organisms with 3 of 400 vegetables (0.75%) still being contaminated.

4. Discussions

Vegetables and especially salads are an important route of transmission of intestinal parasites (helminth and protozoan cysts, ova and larvae) and have been shown to be an important source of food-borne diseases (El Said Said, 2012). In the study presented here, vegetables directly harvested and market "ready to eat" vegetables were found to be contaminated with a variety of parasitic organisms although market "ready to eat" vegetables had a lower percent of infected vegetables. The percent of vegetables directly harvested with parasitic organisms (53.14%; 845/1600) was higher than that reported in other studies from Iran (36.8% (Asadpour et al., 2016);) and from other countries such as West Bengal, India (44.2%; (Gupta et al., 2009), although lower than reported elsewhere in Iran (65% (Gharavi et al., 2002);). In regards to market vegetables, the results in this study (18.2%) also were higher and lower than that reported for other studies in Iran and other countries (Adanir and Tasci, 2013; Asadpour et al., 2016; Balarak et al., 2016). Reasons for differences in the percent of vegetables with parasitic organisms might be due to differences in the vegetables assessed in each study, season of the study and methods of parasite recovery used in the studies (Maikai et al., 2012a). For example, the study by Balarak et al. (2016) examined vegetables in the summer only and focused on leafy vegetables. True differences in percent positive might be due to differences in the farming production systems (fencing, water source, etc.) and post-harvesting handling methods as well as climatic differences. In addition, comparing percent positive to other studies also can be challenging, because it is often not clear if vegetables had only single parasite species present or multiple species present; that is, in the calculation of total contaminated vegetables there is the potential that studies counted a vegetable twice if infected with two different parasite species.

Of the vegetables examined in this study, the majority contaminated with parasitic organisms were leafy vegetables with lettuce (91.07%), leek (90%) and cabbage (67.27%) being highest in field collections and lettuce (55.44%) and leek (45.91%) being highest among those from markets. This agrees with Uga et al. (2009), whose work shows that contamination was high in leafy vegetables followed by root and fruity vegetables. Also, this finding is in accordance with the study of parasitic contamination of lettuce (96%) in Tripoli, Libya reported by Abougrain et al. (2010). Damen et al. (2007) detected contamination in 40% of lettuce samples and 24% in green leafy vegetable in Nigeria. A possible reason for this higher level on leafy vegetables could be that the uneven surfaces and broad leaves lead to more contact with sewage contaminated surface soil (Siyadatpanah et al., 2013). In the case of "ready to eat" leafy vegetables, as well as vegetables with rough surfaces, higher levels might be due to trapped pathogens in the leaves and difficulty with washing. Rahman et al. (2014) indicated that vegetables with a smooth surface such as cucumber had the least occurrence of parasite

Table 4
Association of vegetable contamination with parasitic organisms and fencing, toilets, irrigation and fertilizer.

Variable			Number of vegetables sampled	Number (percentage) of vegetables with each parasite species (%)													Number (percentage) of vegetables with at least 1 parasite
Variables Description		Name of Fields		*E. c*	*G. i*	*C. p*	*F. h*	*D. d*	*T. s*	*H. n*	*A. s*	*T. ct*	*T. cn*	*S. s*	*A. l*	Dog & cat *T. ct* & *T. cn*	
Fence[a] (n = 1600)	Yes	A-H	800	64 (8.0)	68 (8.5)	39 (4.8)	119 (14.8)	113 (14.1)	46 (5.7)	45 (5.6)	24 (3.0)	46 (5.7)	39 (4.8)	74 (10.1)	72 (9.0)	85 (9.2)	392 (49.0)
	No	I–P	800	100 (12.5)	86 (10.7)	74 (9.2)	220 (27.5)	195 (24.3)	121 (15.1)	35 (4.3)	46 (5.7)	239 (29.8)	192 (24.0)	73 (9.1)	76 (9.5)	443 (55.3)	605 (75.6)
Sanitary toilet (n = 1600)	Yes	A-D, I-L	800	75 (9.3)	50 (6.2)	52 (6.5)	149 (18.6)	155 (19.3)	76 (9.5)	15 (1.8)	35 (4.3)	127 (15.8)	115 (14.3)	36 (4.5)	37 (4.6)	254 (31.7)	479 (59.8)
	No	E-H, M-P	800	89 (11.1)	104 (13.0)	61 (0.0)	190 (7.6)	153 (19.1)	91 (11.3)	65 (8.1)	35 (4.3)	158 (19.7)	116 (14.5)	111 (13.8)	111 (13.8)	274 (34.2)	730 (46.2)
Irrigation[a] (n = 1600)	River	A,B,E,F,I,J,M,N	800	50 (6.2)	47 (5.8)	24 (3.0)	157 (19.6)	149 (18.6)	72 (9.0)	9 (1.1)	36 (4.5)	135 (16.8)	128 (16.0)	34 (4.2)	18 (2.2)	263 (32.8)	435 (54.3)
	Wastewater	C,D,G,H,K,L,O,P	800	114 (14.2)	107 (13.3)	89 (11.1)	182 (22.7)	159 (19.8)	95 (11.8)	71 (8.8)	34 (4.2)	150 (18.7)	103 (12.8)	113 (14.1)	130 (16.2)	265 (33.1)	774 (96.7)
Fertilizer[a] (n = 1600)	Animal	B,D,F,H,J,L,N,P	800	95 (11.8)	100 (12.5)	70 (8.7)	262 (32.7)	242 (0.3)	92 (11.5)	42 (5.2)	30 (3.7)	155 (19.3)	95 (11.8)	70 (8.7)	78 (9.7)	250 (31.2)	747 (93.3)
	Chemical	A,C,E,G,I,K,M,O	800	69 (8.6)	54 (6.7)	43 (5.3)	77 (9.6)	66 (8.2)	75 (9.3)	38 (4.7)	40 (5.0)	130 (16.2)	136 (17.0)	77 (9.6)	70 (8.7)	278 (34.7)	462 (57.7)

Abbreviations: E. c: Entamoeba coli cyst, G. i: Giardia intestinalis cyst, C. p: Cryptosporidium parvum oocyst, F. h: Fasciola hepatica egg, D. d: Dicrocoelium dendriticum egg, T. s: Taenia spp. egg, H. n: Hymenolepis nana egg, A. s: Ancylostoma spp. egg, T. ct: Toxocara cati egg, T. cn: Toxocara canis egg, S. s: Strongyloides stercoralis larva, A. l: *Ascaris lumbricoides* egg.

[a] The frequency of the number of vegetables with at least 1 parasite organism was statistically significantly different within each of these variables. That is, frequency with a fence was different from no fence, with river different from wastewater irrigation and animal different from chemical fertilizer, $p < 0.05$.

Table 5

Parasitic contamination of vegetables from fields and “ready to eat” vegetables by season from Tabriz, Iran.

Season	Vegetables obtained from fields			“Ready to eat” vegetables from markets		
	Number of vegetables examined	Positive samples		Number of vegetables examined	Positive samples	
		Number	%		Number	%
Spring	400	219	54.75	317	109	34.38
Summer	475	357	75.15	419	52	12.41
Autumn	400	184	46	221	29	13.12
Winter	325	85	26.15	200	21	10.50
Total	1600	845	52.81	1157	211	18.23

Based on a chi-square, the frequency of the number of vegetables with parasite organisms for the four seasons were statistically significantly different for both field and “ready to eat” vegetables, $p < 0.05$.

Table 6

The impact of washing procedure on number of vegetables contaminated with parasitic organisms.

Washing Procedures	Number of vegetables examined	Positive samples after washing	
		Number	%
Water with two drops of dishwashing liquid	400	160	40
Vinegar	400	120	30
0.95% $Ca(ClO)_2$	400	3	0.75
Lemon juice	400	92	23

Based on a chi-square, the frequency of the number of vegetables with parasite organisms for the four washing systems were statistically significantly different for both field and “ready to eat” vegetables, $p < 0.05$.

organisms. However, in our study presented here cucumber (25%) was higher than leafy vegetables such as dill, mint and savory (5%, 4.54% and 2.50%, respectively); therefore, there might be additional characteristics leading to contamination differences. The high contamination in leek, which has a long cylinder like bundled leaf sheath, seems to be due to direct contact of its leaves with soil (Kozan et al., 2005); however, results with leek are inconsistent with (Asadpour et al., 2016; Fallah et al., 2012; Shahnazi and Jafari-Sabet, 2010; Shahnazi et al., 2009) also finding high levels in leek but with (Al-Binali et al., 2006) only finding 13% to be contaminated.

Based on the best subsets analysis, which considered the 16 cropping systems, no single crop system characteristic could predict contamination levels across the cropping systems. However, there were differences in the number of vegetables contaminated and diversity of parasite species found when each characteristic was considered individually. For example, fencing, which would decrease domestic animal access to the crops, resulted in significantly reduced rates of parasitic contamination compared to fence-free systems. Similar results were reported by a study from Semnan, Iran, which showed that farm fencing can be effective in reducing the presence of parasite eggs from domestic animals on vegetables (Nazemi et al., 2012). Given that many parasites can come from free-roaming domestic animals as well as animal fertilizer, the best assessment of the impact of fencing is with *T. canis* and *T. cati*, which only could have come from free-roaming dogs and cats. In our study, unfenced cropping areas had a contamination rate of 55.3% with these parasites compared to fenced areas which had 9.2%.

A sanitary toilet did not appear to have an important impact on differences in parasitic organisms on the vegetables. *Ascaris lumbricoides*, which is solely of humans, was lower in cropping systems with a sanitary toilet. However, there was even a larger difference in the use of river versus wastewater for irrigation. Overall, cropping systems irrigated with untreated wastewater had a higher parasitic contamination rate than those irrigated with river water (96.7% versus 54.3%). Similar results was reported by Hajjami et al. (2013) who showed that vegetables irrigated with raw wastewater had higher helminth egg contamination levels. In addition, several epidemiological studies around the world have revealed an excess of parasitic infestations associated with raw wastewater reuse in irrigation (Hajjami et al., 2013) and Bryan (1977) reported 3 epidemics of ascariasis in Germany, associated with food contaminated by wastewater.

Animal manure is considered an economical and organic alternative to chemical fertilizer and hence is frequently used (Kumar et al., 2013). However, our study shows that the highest infestation of parasitic contamination was in the vegetable samples collected from cropping systems that used animal manure (93%). Similar results were reported by a study from Malayer, which showed higher parasitic infestation of vegetables in farms which fertilized with animal manure as compared to farms which fertilized with chemical manure (Rahmati et al. (2017). In contrast, Nazemi et al. (2012) reported that there was no statistically significant relationship between fertilizers and the presence of parasites in agriculture fields. This might be due to differences in parasite prevalence and/or management in the livestock. In the study presented here, the impact of animal manure on contamination also is supported by the presence of *Fasciola hepatica* eggs, which was the most single common parasite recovered from vegetables and higher in those treated with manure than all other cropping systems, including ones with no fencing where cattle had access.

Considering seasonal variability, this study indicated that the percent of vegetable samples obtained directly from the fields and market “ready to eat” vegetables with parasite contamination was the highest in summer (75.15%) and spring (34.38%), respectively, and the lowest in winter (26.15% and 10.50%, respectively). Our finding is consistent with previous studies by Kozan et al. (2007) and El Said Said (2012) who reported a higher rate of parasitic contamination in vegetables during warm seasons than those during cold seasons. This could be related to the use of untreated wastewater for irrigation of vegetables during summer and spring (Fallah et al., 2016), although in Tabriz this wastewater is often still used in winter even with crop row tarps. On the contrary, Uga et al. (2009) have reported that the highest parasitic contamination occurred during the winter season because of the more favorable temperature and moisture conditions for parasite organism survival compared to hot dry summer conditions. These differences in studies indicate that seasonality is likely regional, depending on the average summer and winter temperatures. Another factor that can contribute to the seasonality for the field vegetables, is the use of crop row tarps in winter, which potentially could decrease the exposure of the growing vegetables to livestock, dogs and cats, especially in unfenced fields.

The level of parasitic contaminants present in market “ready to eat” vegetables is not only potentially related to the cropping system, but also the post-harvesting processes. Use of contaminated water for washing vegetables, the use of the same container and water to wash different types of vegetables and poor hygienic practices of the personnel handling the vegetables might contribute to higher levels of contamination (Akoachere et al., 2018). The occurrence of these practices were not assessed in this study although, based on the primary origin of the vegetables, it is believed that traditional washing methods were used. Based on the percent of “ready to eat” vegetables with parasitic contaminants compared to those freshly harvested, the cleaning procedures decrease but do not eliminate the contaminants. However, a limitation in this study is that while origin of the “ready to eat” vegetables based on the vegetables, markets and grocers selected is believed to be Tabriz, this could not be confirmed. Therefore, differences might be due to origin as well as washing procedures used.

Regardless of the origin of the vegetables, the presence of parasitic organisms in “ready to eat” vegetables suggest that better washing procedures are needed. In the study presented here, use of a 0.95% $Ca(ClO)_2$ solution, a sanitizer used for washing fresh produce in some food processing systems, resulted in <1% of the vegetables being contaminated. While it might not be feasible in all production systems to wash vegetables with the rigor used in the evaluation of washing methods or with $Ca(ClO)_2$, it might be feasible to target vegetables known to have a higher contamination level such as leafy vegetables. In situations where traditional washing systems cannot be significantly altered and in home environments, the addition of vinegar or lemon juice to the water for washing could decrease the number of contaminated vegetables compared to washing with only dishwashing liquid; however, $\geq$23% still were contaminated.

5. Conclusion

Decreasing the level of parasitic organisms on vegetables requires both decreasing contamination during production and increasing the efficacy of washing methods prior to marketing the vegetables. In this study, parasite contamination levels were explored in relation to crop production system characteristics, season, vegetable type and washing methods. By identifying factors that contribute to higher contamination, efforts to decrease contamination can be focused. Based on the results of this study, use of fencing, river water over wastewater and chemical fertilizer could result in lower contamination during production. The highest level of contamination of vegetables is during the summer, and further studies are needed to determine if seasonal changes in irrigation and fertilizer could have an impact in situations where resources do not permit use of year round chemical fertilizer and cleaner irrigation water. In the case of “ready to eat” vegetables, based on the results presented here, any currently used methods of cleaning before market are insufficient to remove all parasite contaminants. Based on the results of the study comparing washing procedures, $Ca(ClO)_2$ was the most effective and encouraging its use prior to marketing vegetables might have a higher impact on decreasing parasite contaminants over other methods. Lastly, this study found that lettuce and leeks, at harvest and at market, had the highest percent of infection and highest number of species present, suggesting that efforts can be focused on particular vegetables in situations where production system and sanitation changes cannot be implemented for all vegetables. While this study focused on Tabriz, the importance of washing vegetables and use of fences, clean irrigation water and non-manure sources of fertilization in vegetable production can be more broadly applied to other regions with similar production systems.

Declaration of competing interest

The authors declare that there are no conflicts of interest relevant to this study.

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