



Parasitic Infections in Raw Vegetables of Kermanshah, Western Iran and Their Relation with Season and Washing Procedures

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HIGHLIGHTS

- Intestinal parasites were found in 63.7, 49.1, and 36.9% of unwashed, traditionally washed, and standard washed vegetables, respectively.
- A meaningful difference was observed in the contamination rate of samples during various seasons.
- No significant relations were found between contamination rates and types of vegetables.
- Vegetables could be considered as a potential vehicle of food-borne parasites in Kermanshah, Iran.

Article type

Original article

Keywords

Vegetables
Parasites
Risk Factors
Food Safety

Article history

Received: 22 Dec 2016
Revised: 6 Feb 2017
Accepted: 7 March 2017

ABSTRACT

Background: Raw or minimally processed vegetables can be important vehicles of food-borne parasites. The main purpose of the present study was to investigate the parasitic infections in raw vegetables from Kermanshah, Western Iran and their relation with season and washing procedures.

Methods: From January to December 2013, raw vegetable samples were collected from Kermanshah, Western Iran. The samples were divided in three different groups, including unwashed, traditionally washed, and standard washed groups. After preparation of the samples, they were examined microscopically for the presence of parasites. Using Chi-square test, the analysis was carried out by SPSS software for windows (version 16.0).

Results: With regards to washing procedures of vegetable samples, intestinal parasites were found in 63.7, 49.1, and 36.9% of unwashed, traditionally washed, and standard washed samples, respectively showing a statistically significant difference ($p < 0.05$). A meaningful difference ($p < 0.05$) was observed in the contamination rate of samples during various seasons as summer > spring > autumn > winter. However, no significant relationship was found between contamination rates and types of vegetables ($p > 0.05$).

Conclusion: Raw vegetables sold in major markets in Kermanshah, Western Iran could be considered as potential vehicles for transmission of parasitic infections to consumers. Regular education must be applied about standard washing and disinfecting procedures of the raw eaten vegetables to reduce exposure of Iranian population to the vegetable-borne parasites.

Introduction

Fresh raw leafy vegetables are essential part of a healthy and balanced meal owing to its high nutritional compounds, including vitamins, dietary fiber, minerals,

fluid, etc. (Fallah et al., 2016; Losio et al., 2015). Moreover, the tendency for regular consumption of raw or slightly cooked vegetables globally increases as it can decrease risks of a widespread range of human health problems such as cardiovascular diseases, diabetes, stroke, and some types of cancers (Adanir and Tasci, 2013; Van Duyn and Pivonka, 2000).

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To cite: Salavati Z., Chalehchaleh A.A., Rezaei F. (2017). Parasitic infections in raw vegetables of Kermanshah, Western Iran and their relation with season and washing procedures. 4: 37-41.

A downside to the potential health benefits is that raw or minimally processed vegetables without adequate washing, disinfection, and/or cooking can be important vehicles of food-borne parasites (Adenusi et al., 2015; Liu et al., 2013; Rostami et al., 2016; Sánchez et al., 2012). Vegetables can be contaminated during production, collection, transferring, preparation, and processing (Maikai et al., 2012). Contamination may be due to the use of animal or human feces, fecal contaminated soil or wash water, inadequately treated or raw wastewater, untreated night soils used as farm fertilizers and human handling (Adenusi et al., 2015; Fallah et al., 2012). In the past years, a large widespread outbreaks due to parasitic contamination of fresh vegetables, salad crops or fruits has been reported in both developing and industrial countries, suggesting their important roles for transmission of protozoan cysts, oocysts, helminth eggs, and larvae to humans (Adanir and Tasci, 2013; Adenusi et al., 2015; Fallah et al 2016; Rostami et al., 2016). Alteration in consumption trends and practices, production, post-processing, and marketing of food products could be related to the increase in the number of vegetable-borne outbreaks (Sánchez et al., 2012). Morbidity due to the parasitic contamination through the consumption of raw leafy green vegetables has been related to harmful effects such as malnutrition, severe anemia, iron loss as well as intestinal obstruction (Hotez et al., 2008).

Internationally, numerous studies reported the parasitic contamination of raw vegetables and ready-to-eat products in wholesale and retail markets (Adamu et al., 2012; Adanir and Tasci, 2013; Adenusi et al., 2015; Hassan et al., 2012; Losio et al., 2015; Van Duyn and Pivonka, 2000). Some studies were conducted on parasitological quality of vegetables and fruits often eaten raw or semi-cooked in different parts of Iran (Daryani et al., 2008; Fallah et al., 2016; Rostami et al., 2016; Saki et al., 2013). However, based on our knowledge, there is no previously published study on the prevalence of parasitic contamination in raw vegetables purchased from Kermanshah province as the first rank of agricultural regions in West part of Iran. Therefore, the main purpose of the present study was to investigate parasitic infections in raw vegetables from Kermanshah, Western Iran and their relation with season and washing procedures.

Materials and methods

Sample collection

From January to December 2013, raw vegetable samples were randomly collected from five different areas of Kermanshah district, Western Iran. The screened vegetable samples were consisted of basil (*Ocimum basilicum*, n=21), leek (*Allium ampeloprasum*, n=31), dill

(*Anethum graveolens*, n=33), radish (*Raphanus sativus*, n=33), mint (*Mentha spicata*, n=30), coriander (*Coriandrum sativum*, n=33), scallion (*Allium schoenoprasum*, n=25), savory (*Satureja hortensis*, n=14), cress (*Lepidium sativum*, n=32), parsley (*Petroselinum crispum*, n=32), and tarragon (*Artemisia dracunculus*, n=10). The samples (1-1.5 kg of each) were purchased weekly from wholesale and retail markets at different points, put into clean plastic bags, and then immediately transferred to the laboratory.

Sample preparation

For preparation of vegetable samples, firstly the edible parts were separated based on the household practice. For each type of vegetable, the following three groups were considered: the first group was unwashed as a control sample, the second group was washed with traditional washing method, and the third group was washed with standard washing procedure. In the traditional method as a common method used for washing of vegetables in Iran, a portion of 250 g of each sample was weighted and soaked into 1-2 L washing water inside a sink. Thus, mud and dust of the vegetables were removed. Then, each sample was carefully collected, put into a clean basket and rinsed for 1.5-2 min with tap water. Afterward, each sample was removed from the basket, and then the remained washing water was left overnight to take place the sedimentation. According to the standard method recommended by Iranian Ministry of Health and Medical Education, the vegetable samples were firstly washed as described in the traditional method and then soaked into 30 ml of a solution containing 200 ppm of active calcium hypochlorite for approximately 2-3 min, rinsed with washing water (5 min), and collected in a clean basket (Fallah et al., 2012).

Detection of intestinal parasites

Amount of 250 g of each unwashed and washed raw vegetable sample was weighted out into a sterile plastic bag, washed with 250 ml physiological saline solution (0.95% NaCl) and left for about 24 h for sedimentation to take place. After discarding the upper layer, the residue was collected and transferred into a 15 ml conical plastic tube. The suspension was centrifuged at 2000 ×g for 15 min and then the top layer was removed. The resulting supernatant was dissolved in distilled water and centrifuged at 2000 ×g for 15 min. The remaining residue was fixed with 4% formaldehyde and mixed with a drop of Lugol's iodine solution. Subsequently, a drop of each solution was placed on a clean, grease-free glass slide, put a clean cover slip, and examined microscopically for the presence of parasites (eggs, cysts, and larva) using ×10 and ×40 objectives (Adenusi et al., 2015).

Statistical analysis

The analysis of the data obtained from the present investigation was performed by SPSS software for windows (version 16.0, SPSS Inc., Chicago, IL, USA). For this purpose, Chi-square test was used to determine the relationship between prevalence rates of parasitic infection and season as well as washing procedures. Significance level was considered at $p < 0.05$.

Results

With regards to washing procedures of vegetable sam-

ples collected from five different areas of Kermanshah district, Western Iran, intestinal parasites were found in 63.7, 49.1, and 36.9% of unwashed, traditionally washed, and standard washed samples, respectively showing a statistically significant difference ($p < 0.05$) as the detail findings in this regard are indicated in Table 1.

A meaningful difference ($p < 0.05$) was observed in the contamination rate of samples during various seasons as: summer > spring > autumn > winter which is shown in Table 2. However, no significant relationship was found between contamination rates as well as types of vegetables ($p > 0.05$).

Table 1: Various parasitic contamination rates (%) in unwashed, traditionally washed, and standard washed vegetables collected from Kermanshah, Western Iran

Parasite	Unwashed	Traditionally washed	Standard washed
<i>Giardia</i>	6.1	4.9	3.4
<i>Ascaris lumbricoides</i>	30.2	15.2	10.6
<i>Hymenolepis nana</i>	5.1	3.7	1.9
<i>Dicrocoelium</i>	4.4	5.3	3.1
<i>Toxocara</i>	19.7	10.2	7.5
<i>Taenia/Echinococcus</i>	19.0	7.8	5.6
<i>Fasciola</i>	3.1	0.8	1.2
<i>Entamoeba histolytica/dispar</i>	1.4	2.9	1.2
<i>Entamoeba coli</i>	11.5	6.6	7.5
<i>Trichostrongylus</i>	2.7	1.2	2.5

Table 2: Seasonal variability of parasitic contamination rates (%) in vegetable samples collected from Kermanshah, Western Iran

Parasite	Spring	Summer	Autumn	Winter
<i>Giardia</i>	16.7	8.8	4.3	0.0
<i>Ascaris lumbricoides</i>	38.1	45.0	30.1	11.2
<i>Hymenolepis nana</i>	2.4	10.0	6.5	0.0
<i>Dicrocoelium</i>	0.0	13.8	1.1	1.2
<i>Toxocara</i>	11.9	30.0	11.8	22.5
<i>Taenia/Echinococcus</i>	28.6	40.0	8.6	5
<i>Fasciola</i>	9.5	2.5	3.2	0.0
<i>Entamoeba histolytica/dispar</i>	19.0	2.5	1.1	1.2
<i>Entamoeba coli</i>	50.0	11.2	4.3	0.0
<i>Trichostrongylus</i>	2.4	7.5	1.1	0.0

Discussion

Various prevalence rates of different kinds of parasitic infections, including protozoa, cestoda, trematoda, and nematoda have been detected in vegetable samples of the present study which are comparable with some previous reports in the other regions of Iran (Asadpour et al., 2016; Daryani et al., 2008; Ezatpour et al., 2013; Fallah et al., 2012). Also, we found a considerable reduction of parasitic contamination rates in standard washed vegetables comparing with the other groups. Although average parasitic contamination rate in unwashed vegetables found in this work was lower than that of reported from Mazandaran province, Northern Iran (Rostami et al., 2016), but it was higher than the rate of reported from vegetables sold in some other regions of this country such as Shahrekord, South-West Iran (Fallah et al., 2012); Ardebil, North-West Iran (Daryani et al., 2008); and Shiraz, Southern Iran (Asadpour et al., 2016). It has been indicated that the Northern Iran is a highly endemic area for intestinal parasites due to high humidity and warm ambient temperature which is environmental condition favor for the development of transmissible forms of parasites (Omran et al., 2015). However, variation in the average parasitic contamination of raw vegetables could be due to some other factors such as processing and washing method (Fallah et al., 2012). Comparing our findings with similar works in all over the world, the contamination rates observed in the present research were somewhat higher than those of previously reported from other countries such as Turkey (Adanir and Tasci, 2013; Kozan et al., 2005), Libya (Abougrain et al., 2010), Egypt (Hassan et al., 2012), and Nigeria (Adamu et al., 2012). However, Nyarango et al. (2008) revealed that 75.9% of raw vegetable samples in Kenya were contaminated with various parasites which were too higher than that of our study. It seems that variations in prevalence of parasitic contamination of raw vegetables among different studies can be associated with geographical and climate conditions, type of vegetables, sampling season, sample size, parasitological method, etc. (Adenusi et al., 2015). The high parasitic contamination in the current study may be contributed to the uncontrolled application of animal/human manure and night soil as natural fertilizers as well as soil and water supplies contaminated with sewage to irrigate vegetables (Kozan et al., 2005).

In the current investigations, we observed an effective role of standard washing procedure for reduction (but no elimination) of parasitic contamination of vegetables. In agreement with our study, Kozen et al. (2005) reported soil-transmitted helminths contamination in 1.2 and 3.2% of standard as well as traditionally washed vegetables, respectively. In comparison, Fallah et al. (2012) found intestinal parasites in 32.6% unwashed, 1.3% tradition-

ally washed, and 0% standard washed vegetables of Shahrekord, Iran. In spite of the findings of the recent mentioned work, our results indicated that standard washing procedure can not completely remove parasites from the vegetables. It should be noted that the occurrence of parasitic contamination in the standard washed vegetables is probably due to this fact that disinfectant solution is capable of completely killing worm eggs, cysts, and larvae but the remained dead agents in the corresponding vegetables is still detectable via light microscopy (Eraky et al., 2014).

Considering seasonal variability, our results are in agreement with those of reported by other authors from Iran and other countries (Adamu et al., 2012; Adenusi et al., 2015; Eraky et al., 2014; Shahnazi and Jafari-Sabet, 2010; Uga et al., 2009). It can be concluded that the higher degree of parasitic contaminations during the warm seasons seems to be a general phenomenon; because the parasite's eggs shedding by human and animals is higher in warm seasons when compared to cold ones (Al-Megrm, 2010; Daryani et al., 2008; Ezatpour et al., 2013). In addition, in the cold seasons, most days of Kermanshah city is rainy and therefore, helminth eggs on the surface of vegetables may be washed away by rain (Pullan et al., 2014). According to these reasons, seasonal variations could be considered as one of the most important risk factors of parasitic contaminations in raw consumed vegetables.

Conclusion

The results of the present study demonstrated that raw vegetables sold in major markets in Kermanshah, Western Iran could be considered as potential vehicles for transmission of parasitic infections to the consumers. Considering the results, regular education must be applied about standard washing as well as disinfecting procedures of the raw eaten vegetables in order to reduce exposure of Iranian population to the vegetable-borne parasites. Moreover, it seems that one of the best approaches to deal with this problem is to disinfect wastewater used for irrigation of vegetables.

Conflicts of interest

All the authors declared that there was no conflict of interest in this study.

Acknowledgments

The authors acknowledge Razi University, Kermanshah, Iran for financial support. This research was ethically approved by the local institutional review board.

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