Prevalence of parasitic contamination of raw vegetables in Khorramabad, Iran

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A B S T R A C T

It is well known that raw vegetable was considered as an agent for transmission of intestinal parasite and fresh vegetables have an important role in human nutrition. Therefore, the present study was designed to detect the parasite contamination in many common raw vegetables in Khorramabad, Iran. A total of 550 fresh vegetable samples which belonged to spring (275) and winter (275) were randomly collected. All samples were examined according to standard methods for detection of protozoan cyst, oocysts, helminth eggs as well as larva. The findings indicated 52.7% positive for intestinal parasites and the highest contaminated sample was leek 80% in spring and 43.6% in winter. However, the least contaminated samples were green onion (34.5%) in spring and garden cress (10.9%) in winter. Chi-square test indicated the significant difference between contamination in spring when compared to winter (p < 0.002). Our data demonstrated importance of raw vegetables in transmission of some intestinal parasites and highlight the role of raw vegetables in threatening public health.

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1. Introduction

Vegetables are essential part of a healthy human diet owing to their nutritional value. Raw vegetables are great source of vitamins, dietary fiber and minerals; and their regular consumption is associated with a reduced risk of cardiovascular disease, stroke and certain cancers (Van Duyan & Pivonka, 2000). Some vegetables are eaten raw as salad to retain the natural taste and preserve heat labile nutrients. Vegetables can become contaminated with enteric bacterial, viral and parasitic pathogens throughout the process from planting to consumption. The extent of contamination depends on several factors that include, among others, use of untreated water and water supplies contaminated with sewage for irrigation, post-harvest handling and hygienic condition of preparation in food service or home settings (Al-Binali, Bello, El-Shewy, & Abdulla, 2006; Amoah, Drechsel, Abaidoo, & Klute, 2007).

In recent years, there has been an increasing in number of reported cases of food-borne illnesses linked to consuming of fresh vegetables. The consumption of raw vegetables plays a major epidemiological role in the transmission of parasitic food-borne diseases. Intestinal parasites are widely prevented in developing countries, probably due to poor sanitation and inadequate personal hygiene (Al-Binali et al., 2006). Several studies in different parts of the world showed that the vegetables can be agent for transmission of protozoan cysts, oocysts, helminthes eggs and larvae (Al Salem & Tarazi, 1992; Anuar & Ramachandran, 1977; Bailenger, 1962). This problem is becoming increasing concern because of the expanding number of susceptible people (i.e., the elderly and the immunocompromised) more extensive produce trade across international borders, and change in national and international policies concerning food safety (Akhlaghi & Oormazdi, 2000).

Up to our knowledge, there are limited studies on the possible contamination of freshly eaten vegetables in Iran and referring to existing scientific literature, no previous surveys have been conducted to evaluate the presence of parasitic contamination in vegetables in Khorramabad. Therefore, this study was designed to detect the parasitic contamination in some common green vegetables used for raw consumption in Khorramabad, Iran.
### Table 1
Distribution of eggs and cysts of parasites in considered vegetables from spring.

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>No.</th>
<th>Pathogenic parasites</th>
<th>Nonpathogenic parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Giardia cys. no. (%)</td>
<td>Ascaris eggs. no. (%)</td>
</tr>
<tr>
<td>Leek</td>
<td>55</td>
<td>5 (9.1)</td>
<td>3 (5.5)</td>
</tr>
<tr>
<td>Green onion</td>
<td>55</td>
<td>0 (0)</td>
<td>6 (10.9)</td>
</tr>
<tr>
<td>Radish</td>
<td>55</td>
<td>2 (3.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Garden cress</td>
<td>55</td>
<td>6 (11)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>Mint</td>
<td>55</td>
<td>3 (5.3)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>Total</td>
<td>275</td>
<td>16 (5.8)</td>
<td>13 (4.7)</td>
</tr>
</tbody>
</table>

### 2. Materials and methods

#### 2.1. The study area

The study was carried out in Khorramabad, the capital and largest urban center in Lorestan state, west of Iran between winter and spring 2011. Khorramabad is at a height of about 1125 m above sea level with a latitudinal position of latitude 33° 26' N and longitude 45° 17' E. The city has a population of 354,855 based on 2011 census ("Statistics in Iran, 1390. population census", 2011). The temperature mean value of Khorramabad is 17.60 °C, however, during the cooler periods (November and March) the average temperature drops to about 5 °C, relative humidity mean value is 46.08% and precipitation mean monthly value is 42.74 mm ("Available from this site: http://www.climate-charts.com/Locations/i/IR40782.php.").

#### 2.2. Sampling

A total of 550 samples of fresh vegetables (275 samples in spring and 275 samples in winter), including Leek (Allium porrum), Green onion (Allium ascalonicum), Mint (Mentha piperita), Radish (Raphanus sativus), Garden cress (Lepidium sativum) were randomly collected in batches of five per day from retailers at different points. During spring vegetables available in Khorramabad markets are from the fields around the Khorramabad district; but due to the unfavorable climatic conditions in winter, the vegetables are imported from Khuzestan province (south of Iran) at that season.

#### 2.3. Procedure for sample preparation & determination of parasites

The vegetables (250 g) were collected and weighted into sterile nylon bags and transported for analysis to Parasitological Laboratory of the Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran. Sample washed by vigorous shaking with 1 L of physiological normal saline (0.95% NaCl). The washing water was then left for about 12 h for sedimentation to take place. The top layer was discarded and the remaining washing water was centrifuged at 2000 rpm for 15 min according to previous report with modification (Uga et al., 2009), the supernatant was discarded and the sediment carefully collected. The sediment was mixed and examined as follow:

1. Direct smear: a drop of the sediment was applied on the center of a clean grease-free slide. A clean cover slip was placed gently to avoid air bubbles and over flooding. The preparation was examined under a light microscope using ×10 and ×40 objectives (two for each sample) (Garcia & Bruckner, 1993).

2. Lode smears: a drop of the sediment was mixed with a drop of Lugol’s iodine solution and examined as in direct smear (two for each sample) (Garcia & Bruckner, 1993). Smears were used for detection of parasitic eggs, cysts and larva. Parasites found under the light microscope were identified as described by Downes (Downes & Ito, 2001).

#### 2.4. Data analysis

Statistical analyses were carried out using Chi-square test of the SPSS software version 16 for Windows (SPSS Inc., Chicago, IL, USA) to compare the rate of contamination of vegetables among different seasons. A P-value < 0.05 was considered statistically significant.

### 3. Results

In the present study, five pathogenic and four nonpathogenic parasites were found in contaminated vegetables. Tables 1 and 2 summarize the results of the presence of various parasites such as nonpathogenic parasites (Free-living larvae, Entamoeba coli cyst, Iodamoeba butschlii cyst and Endolimax nana cyst) and pathogenic parasites (A. lumbricoides eggs, Enterobius vermicularis eggs, Giardia spp. cysts, Strongyloides stercoralis eggs and Fasciola hepatica eggs) on some raw vegetables (Leek, Green onion, Radish, Garden cress, Mint). From the total samples in spring, 35.3% were being contaminated with the nonpathogenic parasites and 17.5% were found to be contaminated with the pathogenic parasites (Table 1). In contrast, 21.1% were found to be contaminated vegetables with the nonpathogenic parasites in winter and 5.5% were found to be contaminated with the pathogenic parasites (Table 2).

### Table 2
Distribution of eggs and cysts of parasites in considered vegetables from winter.

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>No.</th>
<th>Pathogenic parasites</th>
<th>Nonpathogenic parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Giardia cys. no. (%)</td>
<td>A. lumbricoides no. (%)</td>
</tr>
<tr>
<td>Leek</td>
<td>55</td>
<td>1 (1.8)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Green onion</td>
<td>55</td>
<td>0 (0)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Radish</td>
<td>55</td>
<td>0 (0)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Garden cress</td>
<td>55</td>
<td>2 (3.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mint</td>
<td>55</td>
<td>0 (0)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Total</td>
<td>275</td>
<td>3 (1.1)</td>
<td>3 (1.1)</td>
</tr>
</tbody>
</table>
In spring, the highest number of contaminated vegetables with the pathogenic & nonpathogenic parasites was detected in Leek (*Allium porrum*) (80%), while the lowest number of contaminated samples was detected in Green onion (*Allium ascalonicum*) (34.5%). In winter, the highest number of contaminated vegetables was detected in Leek (43.6%) and the lowest number of contaminated samples was detected in garden cress (10.9%). The highest rate of parasitic contamination in vegetables was found in spring (52.8%). Statistical analysis revealed that the rate of contamination in spring was significantly higher than winter ($p < 0.002$).

4. Discussion

The consumption of raw vegetables plays an important role in the transmission of parasitic contaminations (Anuar & Ramachandran, 1977). Recovery of parasites from vegetables used as the source of contamination may be helpful in indicating the incidence of intestinal parasites among a community. This study showed a considerably high level (52.8% in spring and 26.2% in winter) of contamination of green vegetables with intestinal parasites in Khorramabad.

The 79% contamination rate in this study is high compared to other studies such as the 19.4% and 31.7% in Egypt (El Said Said, 2012; Hassan, Farouk, & Abdul-Ghani, 2012), 32.6% and 71% in Iran (Daryani, Ettehad, Sharif, Gorbani, & Ziae, 2008; Fallah, Pirali-Kheirabadi, Shirvani, & Saei-Dehkordi, 2011), 58% in Libya (Abougrain, Nahai, Madi, Saied, & Ghenghesh, 2010), 16.4% in Saudi Arabia (Al-Megrm, 2010). Examination of vegetable samples in Kenya revealed also high rate of contamination (75.9%) (Nyarango, Aloo, Kabiru, & Nyanchonghi, 2008).

In this study, Leek (80%) and Garden cress (54.5%) being the most contaminated vegetable in spring. In Abha, Saudi Arabia, parasitological contamination was reported to be 13% in leek (Al-Binali et al., 2006). In contrast, a previous study reported that 60% of leek had been contaminated with parasites in an evaluation study of the edible vegetables in Qazvin, Iran (Shahnazi & Jafari-Sabet, 2010).

Eggs of *A. lumbricoides* were detected in 5.8% of vegetables examined being the predominant pathogenic parasite in the present work. The highest contamination with this parasite was detected in Green onion samples (12.7%). The rate of contamination with Ascaris eggs concurs with other studies in Iran. It was 2% in Ardabil (Daryani et al., 2008), 2.5% in Jiruf (Zohour & Molazadeh, 2001) and 2.3% in Qazvin (Shahnazi & Jafari-Sabet, 2010). 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 is associated with the high fertility of these parasites (Klapek & Borecka, 2012). Eggs of *A. lumbricoides* may survive in the external environment and maintain their invasiveness for up to 6 years (Klapek & Borecka, 2012).

In the present investigation, *Giardia* spp. cysts were detected in 6.9% of the total vegetable samples. In Egypt, *Giardia* spp. cysts were detected in 3.7% of different vegetables (El Said Said, 2012). A previous survey from Saudi Arabia reported the *Giardia* spp. cysts in 31.6% of leafy vegetables examined (Al-Megrm, 2010). The finding of this study is almost the same to previous reports in Iran. It was 8.2% in Shahrekord (Fallah et al., 2011), 14% in Jiruf (Zohour & Molazadeh, 2001), 6.5% in Tehran (Gharavi, Jahan, & Rotki, 2002), 9% in Ardabil (Daryani et al., 2008) and 4% in Qazvin (Shahnazi & Jafari-Sabet, 2010).

We detected cyst of *E. coli* in 11.3% of total vegetable samples studied. A survey in Shahrekord, Iran showed that 9.2% of vegetables were contaminated with *E. coli* cyst (Fallah et al., 2011). Despite being nonpathogenic, the presence of *E. coli* cyst denotes the fecal contamination of vegetables (Fallah et al., 2011).

Herein, we did not detect *Toxocara* spp. In consistent with previous reports, it was 19.2% in Poland (Klapek & Borecka, 2012), 19% in Egypt (El Said Said, 2012), 3.3% in Shahrekord (Fallah et al., 2011) and 1.5% in Ankara (Kozen, Gonenc, Sarimehmetoglu, & Aycicek, 2005). The source of the eggs of *Toxocara* spp. may be domestic animals (dogs and cats), and wild animals (foxes, wolves) (Klapek & Borecka, 2012). Lack of *Toxocara* spp. in this study indicates that these animals were not traveling on land under cultivation of vegetables in this area. In addition, several factors may contribute to such differences between present results with other similar studies. These may include; geographical location, type and number of samples examined, methods used for detection of the intestinal parasites, type of water used for irrigation and post-harvesting handling methods of such vegetables. Different laboratory techniques may also contribute to recovery of different parasites since some procedures can either float or sediment the parasites. Iran is among the areas that have significant parasitic infections and therefore identify the sources of parasitic infections, method of spread and transmission, method of prevention are certain health priorities (Sayyari, Imanzadeh, Bagheri Yazdi, Karami, & Yaghoobi, 2005). Since the high Zagros Mountains are in Lorestan and livestock in this region is very prosperous, so the probability of farm land with livestock waste pollution is high. Our finding is consistent with previous studies that reported higher rate of parasitic contamination of vegetables during warm seasons than those during cold seasons (Al-Megrm, 2010; Daryani et al., 2008). It has been determined that the excretion of parasite eggs to environment by human or animals is higher in warm seasons when compared to cold seasons (Eslami, Rahbari, Ranjbar Bahadori, & Kamal, 2003).

5. Conclusion

These findings may have important implications for global food safety and emphasize the importance of raw vegetables in threatening public health by transmission of intestinal parasites to humans in Khorramabad. The local health and environmental authorities should improve the sanitary conditions in the areas where the vegetables are cultivated. Proper treatment of waste water used for irrigation of vegetables should be implemented. More researches are needed to do surveys of parasitic contamination in green vegetables in Khorramabad including different districts. Likewise, other researches must be performing to evaluate the level of contamination of irrigation water and soil in which green vegetables are cultivated.

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