Evaluation of Parasitic Pollution in Fresh Unwashed Herbs Sold in Izeh City, Iran

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Abstract

Background: Fresh herbs are a crucial part of a good diet. However, they can easily become contaminated with food-borne pathogens. Eating fresh unwashed or improperly washed herbs has a major role in transmission of some important parasitic diseases.

Objectives: This study was carried out to evaluate parasitic pollution in fresh unwashed herbs sold in Izeh city, Iran.

Materials and Methods: Four hundred samples of fresh unwashed herbs were purchased from a distributor in Izeh city during January 2014 to April 2015. Detergent solution was added to 240 g of each sample. The mixture was shaken and washed solution was filtered through 0.2 µm bottle-top filter. The filtrate was discarded and filter was placed in a 50 mL conical screw cap tube. Then, the detergent solution was added. Following vortex, filter was discarded. The sediment was examined by light microscope after centrifuge. Finally, data descriptive analyses were carried out.

Results: Parasitic pollution was detected in 3.5% of the examined samples, including Cryptosporidium spp. oocyst (1.5%), Giardia spp. cyst (1%), Blastocystis spp. cyst (0.5%) and Taenia spp. egg (0.5%).

Conclusions: Parasitic contamination of fresh herbs sold in Izeh may pose a health risk to consumers if eaten as unwashed or improperly washed.

Keywords: Foodborne Diseases, Vegetables, Eating, Iran

1. Background

Fresh herbs have a high consumption rate in Iran. The sources of herbs contamination are usually animals or humans (1). Fresh herbs can become contaminated with parasitic pathogens throughout the process of planting to consumption (2). Increasing trends in many countries toward eating raw vegetables increase the likelihood of food-borne parasitic infections (3). Evaluation of parasitic pollution in fresh herbs is important because of increasing number of susceptible individuals (3). Many researchers studied the prevalence of parasitic pollution in vegetables in recent years such as Alqumber (4), Sunil et al. (5), Benti et al. (6), Steele et al. (7), Tram et al. (8), Hassan et al. (9), Deedu et al. (10), Ebrahimzadeh et al. (11), Ezatpour et al. (12), Nazemi et al. (13), Malakootian et al. (14), Shahnazi et al. (15), Saki et al. (16), Garedaghi et al. (17), and Dixon et al. (18) Many of mentioned studies carried out in Iran. Nevertheless, the prevalence of parasitic contamination in fresh herbs and other kind of vegetables sold in some cities of Iran is still unknown.

2. Objectives

Updated information on parasitic contamination in fresh herbs sold in Izeh is required to help the local health authorities to take right actions to improve the quality of such products. This study was performed to evaluate possible parasitic pollution in fresh unwashed herbs sold in Izeh, Iran during January 2014 to April 2015.

3. Materials and Methods

3.1. Sample Collection

A total of 400 samples of fresh unwashed herbs were purchased from a distributor in Izeh, during January 2014 to April 2015. Each sample consisted of leek (Allium porrum), cress (Lepidium sativum), basil (Ocimum basilicum), mint (Mentha haplocalis), common purslane (Portulaca oleracea), savory (Satureja hortensis), radish (Raphanus sativus), and scallion (Allium fistulosum). Two hundred and forty grams of each sample including all mentioned herbs (30 g for each kind) weighed and used for sample preparation.

3.2. Sample Preparation

Each sample was placed in a 500 mL Erlenmeyer flask. Then, detergent solution (containing 10 mM Phosphate-buffered saline (PBS) with 0.1% sodium dodecyl sulfate (L3771 Sigma-Aldrich, USA), and 0.1% Tween-20 (PI379 Sigma-Aldrich, USA) were added. The volume was adjusted to 500 mL with detergent solution. The content was slightly...
mixed, then shaken for 1 hour on an automatic shaker (Wise Cube, Wisd Laboratory Equipment, Germany)\(^{(19, 20)}\). Next, herbs were removed by sieve \(^{(8, 21)}\). The washing solution was filtered through 0.2 µm filter (Z222593 Aldrich, Zap Cap bottle-top filter, USA). The filtrate was discarded. The filter was removed from funnel and placed in a 50 mL conical screw cap tube. Then, the detergent solution was added. The content of the tube was agitated by vortex for 60 seconds. After vortex, the filter was discarded. Finally, the tube was centrifuged at 10,000 x g for 5 minutes. The sediment was washed by PBS and used as the sample for detection of parasites.

### 3.3. Parasite Detection

Samples were examined by light microscope (BH2 Series, Olympus, Japan)\(^{(9)}\). Modified Ziehl-Neelsen acid-fast stain was applied for staining of coccidian protozoa oocysts \(^{(10)}\).

### 3.4. Statistical Analysis

Prevalences of parasites were calculated by dividing the number of positive samples by the total number of samples \(^{(22)}\).

### 4. Results

Parasitic contamination was detected in 14 samples. Prevalence of each parasite is shown in Table 1. Microscopic pictures of detected parasites are demonstrated in Figure 1. Cryptosporidium spp. was the most frequent parasitic contamination found in the present study (Table 1). Meanwhile, Taenia spp. and Blastocystis spp. had the lowest prevalence (Table 1).

| Parasite           | Positive Sample\(^{a}\) |
|--------------------|------------------------|
| Blastocystis spp. cyst | 2 (0.5)                  |
| Taenia spp. egg     | 2 (0.5)                  |
| Giardia spp. cyst   | 4 (1)                   |
| Cryptosporidium spp. oocyst | 6 (1.5)              |
| Total               | 14 (3.5)                |

\(^{a}\) Data are presented as No. (%).

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**Figure 1.** Detected Parasites in Examined Fresh Herbs (Original)

| Parasite | Image |
|----------|-------|
| Blastocystis spp. cyst | ![Image](Blastocystis.jpg) |
| Taenia spp. egg | ![Image](Taenia.jpg) |
| Cryptosporidium spp. oocyst | ![Image](Cryptosporidium.jpg) |
| Giardia spp. cyst | ![Image](Giardia.jpg) |
5. Discussion

In developing countries, parasitic pollution is common (2). Consumption of fresh vegetables is one of transmission routes of food-borne parasitic diseases (2) and determining sources of parasitic infections is crucial for preventive programs. The current study showed that fresh herbs were contaminated with important parasites in Izeh city, and the overall prevalence of parasitic contamination was 3.5%. Contamination rate in the current study was low compared to other studies on vegetables in Iran. In several studies on unwashed vegetables, different rates of parasitic contamination were reported in different part of the world such as 2.7% in India (5), 3.5% in Nigeria (23), 5.9% in Turkey (21), 15.5% in Ahvaz, Iran (16), 16% in Saudi Arabia (24), 19.4% in Egypt (9), 29.6% in Kerman, Iran (14), 31.2% in Pakistan (25), 32.6% in Shahrekord, Iran (1), 37% in Golestan, Iran (26), 38% in Semnan, Iran (13), 50.3% in Vietnam (27), 52.7% in Khoramabad, Iran (12), 56.7% in Ardabil, Iran (3), 57.8% in Ethiopia (28), and 58% in Tabriz, Iran (47). Detected parasites in the present study may be considered as pathogenic agents for human, especially in the cases of Cryptosporidium spp. and Taenia spp. In this study, 0.5% of the examined vegetables were found contaminated with Blastocystis spp. Meanwhile, Almegrin in Saudi Arabia detected Blastocystis spp. at the rate of 17.1% in unwashed vegetables (24). Also, Aziznia et al. in Ilam, Iran found Blastocystis spp. in unwashed vegetables but they did not report its prevalence (29). Prevalence of Cryptosporidium spp. in the current study (1.5%) is not in line with the results of other studies such as Dixon et al. (5.9%) (18), Bahadori et al. (6.6%) (30), Tefera et al. (12.8%) (28), Duedu et al. (16.9%) (10), Razavi et al. (23.5%) (31), El Said Said (29.3%) (22) and Le Quy Nh Chau et al. (47.22%) (27). In the present study, Cryptosporidium spp. was the most prevalent parasitic contamination, also Giardia spp. cysts were detected in 1% of the total samples. This finding is approximately consistent with the findings of Dixon et al. (18), Shahnazi et al. (15), Malakootian et al. (14) and Alade et al. (33). However, the prevalence of Giardia spp. in this study does not correlate with the findings of Haq et al. (4.4%) (25), Duedu et al. (5.5%) (10), El Said Said (6.7%) (32), Esboei et al. (6.8%) (26), Ezatpour et al. (6.9%) (12), Garedaghi et al. (7%) (17), Tefera et al. (7.5%) (28), Daryani et al. (7.8%) (3), Ebrahimzadeh et al. (8.1%) (11), Fallah et al. (8.2%) (1), Abougrain et al. (10%) (2), and Saki et al. (13.3%) (16). Giardia spp. may infect humans and cause giardiasis (34). In this study, Taenia spp. was found in 0.5% of all samples which is consistent with the result of Adamue et al. (23), while does not correlate with study results of Belyani et al. (1%) (35), Adenusi et al. (1.2%) (20), Shahnazi et al. (1.8%) (15), Haq et al. (5.1%) (25), Fallah et al. (9.2%) (1), Ebrahimzadeh et al. (13.1%) (11), Daryani et al. (14.1%) (3), Al-Megrin (19.7%) (24), and Abougrain et al. (22%) (2). The differences in the study results may be due to epidemiological factors.

Using filtration method for parasite isolation was the strength of the current study. In all previous studies, researchers used sedimentation techniques for their first stage isolation of parasites from vegetables (the washing water of each sample was left for hours for sedimentation to take place) (2). In the present study, filtration method was used which need shorter time for isolation of possible parasitic contamination. In the current study, microscope was the only tool used for the identification of parasites and it was a weak point in the study. Regarding the results, it is obvious that fresh herbs sold in Izeh city are contaminated with pathogenic parasites. Further studies in larger scale are recommended.

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