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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), Duedu 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 kopetdaghensis*), 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 (P1379 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).

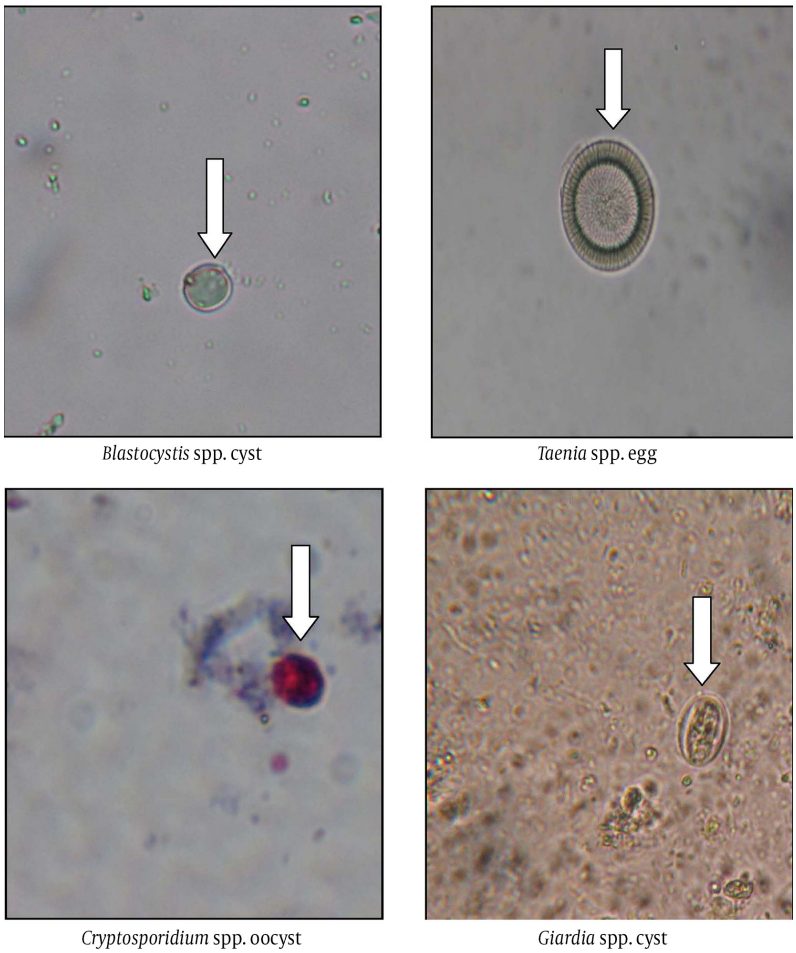
4. Results

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).

Table 1. Prevalence of Parasitic Contamination in 400 Examined Samples of Fresh Herbs

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. (%).



Blastocystis spp. cyst | *Taenia* spp. egg

Cryptosporidium spp. oocyst | *Giardia* spp. cyst

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

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 (17). 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%) (32) and Le Quynh 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 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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References

1. Fallah AA, Pirali-Kheirabadi K, Shirvani F, Saei-Dehkordi SS. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Food Control.* 2012;**25**(2):617–20. doi: 10.1016/j.foodcont.2011.12.004.
2. Abougrain AK, Nahaisi MH, Madi NS, Saied MM, Ghenghesh KS. Parasitological contamination in salad vegetables in Tripoli-Libya. *Food Control.* 2010;**21**(5):760–2. doi: 10.1016/j.foodcont.2009.11.005.
3. Daryani A, Ettehad GH, Sharif M, Ghorbani L, Ziaei H. Prevalence of intestinal parasites in vegetables consumed in Ardabil, Iran. *Food Control.* 2008;**19**(8):790–4. doi: 10.1016/j.foodcont.2007.08.004.
4. Alqumber MA. Parasitic Contamination in Raw Vegetables in Cities of the Sarawat Mountain Range of Saudi Arabia. *Life Sci J.* 2014;**11**(9):707–11.
5. Sunil B, Thomas DR, Latha C, Shameem H. Assessment of parasitic contamination of raw vegetables in Mannuthy, Kerala state, India. *Vet World.* 2014;**7**(4):253–6.
6. Benti G, Gemechu F. Parasitic contamination on vegetables irrigated with Awash River in selected farms, Eastern Showa, Ethiopia. *J Parasitol Vector Biol.* 2014;**6**(7):104–10.
7. Steele M, Unger S, Odumeru J. Sensitivity of PCR detection of Cyclospora cayetanensis in raspberries, basil, and mesclun lettuce. *J Microbiol Methods.* 2003;**54**(2):277–80. doi: 10.1016/s0167-7012(03)00036-8. [PubMed: 12782383]
8. Tram NT, Hoang LM, Cam PD, Chung PT, Fyfe MW, Isaac-Renton JL, et al. Cyclospora spp. in herbs and water samples collected from markets and farms in Hanoi, Vietnam. *Trop Med Int Health.* 2008;**13**(11):1415–20. doi: 10.1111/j.1365-3156.2008.02158.x. [PubMed: 18983280]
9. Hassan A, Farouk H, Abdul-Ghani R. Parasitological contamination of freshly eaten vegetables collected from local markets in Alexandria, Egypt: A preliminary study. *Food Control.* 2012;**26**(2):500–3. doi: 10.1016/j.foodcont.2012.01.033.
10. Duedu KO, Yarnie EA, Tetteh-Quarcoo PB, Attah SK, Donkor ES, Ayeh-Kumi PF. A comparative survey of the prevalence of human parasites found in fresh vegetables sold in supermarkets and open-aired markets in Accra, Ghana. *BMC Res Notes.* 2014;**7**(1):836. doi: 10.1186/1756-0500-7-836. [PubMed: 25424391]

11. Ebrahimzadeh A, Jamshidi A, Mohammadi S. The Parasitic Contamination of Raw Vegetables Consumed in Zahedan, Iran. *Health Scope*. 2013;**1**(4):205–9. doi: 10.5812/jhs.8209.
12. Ezatpour B, Chegeni AS, Abdollahpour F, Aazami M, Alirezaei M. Prevalence of parasitic contamination of raw vegetables in Khorramabad, Iran. *Food Control*. 2013;**34**(1):92–5. doi: 10.1016/j.foodcont.2013.03.034.
13. Nazemi S, Raei M, Amiri M, Chaman R. Parasitic contamination of raw vegetables in Shahroud, Semnan. *Zahedan J Res Med Sci*. 2012;**14**(8):84–6.
14. Malakootian M, Hoseini M, Bahrami H. Investigation of parasitic contamination of vegetables consumed in Kerman city. *Biomonth J Hormozgan U Med Sci*. 2008;**13**(1):55–62.
15. Shahnazi M, Jafari-Sabet M. Prevalence of parasitic contamination of raw vegetables in villages of Qazvin Province, Iran. *Foodborne Pathog Dis*. 2010;**7**(9):1025–30. doi: 10.1089/fpd.2009.0477. [PubMed: 20491596]
16. Saki J, Asadpoori R, Khademvatan S. Prevalence of Intestinal Parasites in Vegetables Consumed in Ahvaz, South West of Iran. *J Med Sci*. 2013;**13**(6):488–92. doi: 10.3923/jms.2013.488.492.
17. Garedaghi Y, Farhang HH, Pooryagoob S. Parasitic Contamination of Fresh Vegetables Consumed in Tabriz, Iran. *Res J Biol Sci*. 2011;**6**(10):518–22. doi: 10.3923/rjbsci.2011.518.522.
18. Dixon B, Parrington L, Cook A, Pollari F, Farber J. Detection of Cyclospora, Cryptosporidium, and Giardia in ready-to-eat packaged leafy greens in Ontario, Canada. *J Food Prot*. 2013;**76**(2):307–13. doi: 10.4315/0362-028X.JFP-12-282. [PubMed: 23433379]
19. Lass A, Pietkiewicz H, Szostakowska B, Myjak P. The first detection of Toxoplasma gondii DNA in environmental fruits and vegetables samples. *Eur J Clin Microbiol Infect Dis*. 2012;**31**(6):1101–8. doi: 10.1007/s10096-011-1414-8. [PubMed: 21948336]
20. Adenusi AA, Abimbola WA, Adewoga TO. Human intestinal helminth contamination in pre-washed, fresh vegetables for sale in major markets in Ogun State, southwest Nigeria. *Food Control*. 2015;**50**:843–9. doi: 10.1016/j.foodcont.2014.10.033.
21. Kozan E, Gonenc B, Sarimehmetoglu O, Aycicek H. Prevalence of helminth eggs on raw vegetables used for salads. *Food Control*. 2005;**16**(3):239–42. doi: 10.1016/j.foodcont.2004.02.005.
22. Maikai BV, Elisha IA, Baba-Onoja EBT. Contamination of vegetables sold in markets with helminth eggs in Zaria metropolis, Kaduna State, Nigeria. *Food Control*. 2012;**28**(2):345–8. doi: 10.1016/j.foodcont.2012.05.035.
23. Adamu NB, Adamu JY, Mohammed D. Prevalence of helminth parasites found on vegetables sold in Maiduguri, Northeastern Nigeria. *Food Control*. 2012;**25**(1):23–6. doi: 10.1016/j.foodcont.2011.10.016.
24. Al-Megrin WAI. Prevalence Intestinal Parasites in Leafy Vegetables in Riyadh, Saudi Arabia. *Int J Trop Med*. 2010;**5**(2):20–3. doi: 10.3923/ijtmed.2010.20.23.
25. Haq S, Maqbool A, Khan UJ, Yasmin G, Sultana R. Parasitic Contamination of Vegetables Eaten Raw in Lahore. *Pak J Zool*. 2014;(46):1303–9.
26. Esboei BR, Pagheh A, Fakhar M, Pagheh S, Dadimoghadam Y. Parasitic Contamination of Consumed Vegetables in Golestan Province, 2012. *Med Lab J*. 2014;**8**(3):82–9.
27. Le Quynh, Chau H, Trung Thong H, Van Chao N, Hoang Son Hung P, Van Hai V, Van An L, et al. Microbial and Parasitic Contamination on Fresh Vegetables Sold in Traditional Markets in Hue City, Vietnam. *J Food Nutr Res*. 2014;**2**(12):959–64. doi: 10.12691/jfnr-2-12-16.
28. Tefera T, Biruksew A, Mekonnen Z, Eshetu T. Parasitic contamination of fruits and vegetables collected from selected local markets of Jimma town, southwest Ethiopia. *Int Sch Res Not*. 2014:1-7. [PubMed: 25546904]
29. Aziznia S, Varharam H, Aziznia H, Hoseini M, Dastjerdi HB, Mousavi SR. Evaluation of the effect of Prosopis Fracta root extract in parasitic disinfection of vegetables in Ilam province. *Food Sci Technol*. 2014;**2**(6):95–103.
30. Bahadori SR, Mostoophi A, Shemshadi B. Study on Cryptosporidium contamination in vegetable farms around Tehran. *Trop Biomed*. 2013;**30**(2):193–8. [PubMed: 23959484]
31. Razavi SM, Nasirinasab-Rafsanjani M, Bahrami S. A study on Cryptosporidium contamination in lettuce collected from different areas in Shiraz. *J Shahrekord Univ Med Sci*. 2010;**12**(2):44–50.
32. El Said Said D. Detection of parasites in commonly consumed raw vegetables. *Alexandria J Med*. 2012;**48**(4):345–52. doi: 10.1016/j.ajme.2012.05.005.
33. Alade GO, Alade TO, Adewuyi IK. Prevalence of Intestinal Parasites in Vegetables Sold in Ilorin, Nigeria. *Am Eur J Agric Environ Sci*. 2013;**13**(9):1275.
34. Dawson D. Foodborne protozoan parasites. *Int J Food Microbiol*. 2005;**103**(2):207–27. doi: 10.1016/j.ijfoodmicro.2004.12.032. [PubMed: 16083823]
35. Belyani S, Saeidi ASL MR. Survey of parasitic contaminations in vegetables in tehran province (before and after washing). *J Innov Food Sci Technol*. 2009;**1**(1):15–24.