Parasitic Contamination of Raw Vegetables Consumed in Hamadan, West of Iran During 2017-2018

Monir Taherimoghaddam¹, Faeze Foroughi-Parvar²*, Manizheh Kashinahanji², Mohammad Matini²

¹Student Research Committee, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
²Department of Medical Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Abstract
Background: Healthy diets including fruits and vegetables reduce the risk of chronic diseases. This study was aimed at detecting parasitic contamination caused by consuming raw vegetables sold in Hamadan, west of Iran.
Methods: A total of 380 vegetable samples consisting of parsley, coriander, basil, savory, mint, cress, tarragon, leek, radish and spring onion were evaluated microscopically after conducting sedimentation and flotation from April 2017 to March 2018.
Results: Parasites were detected in 95 out of 380 of vegetable samples (25%). The organisms identified included Entamoeba coli (8.15%), Ascaris lumbricoides (7.1%), Chilomastix mesnili (5%), and Giardia lamblia (1.5%), Blastocystis hominis (0.78%), Toxocara canis (0.26%) and Hymenolepis nana (0.26%). High level of contamination was obtained for parsley (5.52%) while radish was the least contaminated (0.78%). Vegetable contamination was higher in spring and summer (20.6%) (P<0.0001). Samples collected from vegetable fields irrigated by well water supply showed low parasitic contamination (28.5%) compared to those from farms irrigated by surface or sewage water (91%) (P<0.0001).
Conclusions: The results emphasize the potential role of raw vegetables consumption in transmission of pathogenic organisms. Besides, it was highlighted that irrigation of vegetables by surface water could be the source of vegetable contamination with human intestinal parasites.
Keywords: Food Contamination, Parasites, Vegetables, Iran

Background
Healthy diet including fruits and vegetables reduces the risk of chronic disease such as heart disease, cancer, stroke and diabetes. A broad range of nutrients, phytochemicals, vitamins and minerals are provided through adhering to the diets containing the fruits and vegetables (1). Since the vegetables are served uncooked, without any processing, the contamination of these products seems very important. They can transfer pathogens from planting through consuming process (2). Vegetables will be contaminated with viral, bacterial or parasitological pathogens from different sources, such as human feces and manure that are widely applied as soil fertilizers. Untreated sewage is also used as a source of agricultural water in some regions (3). Foodborne disorders have attracted the attention of public health authorities across the world, as the World Health Organization (WHO) has reported 31 bacteria, viruses, parasites and toxins as the causes of food borne diseases particularly in low socio-economic regions in the world (4). Enteric outbreaks have been reported following consumption of tomatoes, spinach, lettuce and seed sprouts (3). Hamadan province is located in a mountainous region in west of Iran. Vegetables are provided by local farmers in spring and summer but in autumn and winter vegetables are imported from relatively hot regions such as Khuzestan province due to the cold weather of Hamadan province.

Objectives
The present study was conducted to investigate the prevalence of parasitic contamination caused by consumption of raw vegetables as potential source of parasite dissemination.

Material and Methods
Sampling
This study was carried out in Hamadan, west of Iran. The city is located between 34°8’ latitude north and 48°5’ longitude east, and is 1810 m above the sea level, with a population of 676105 according to 2016 Iranian Population and Housing Census (5). This study was carried out from April 2017 to March 2018. For this purpose, first sampling was done during spring and summer and the second sampling was carried out over the autumn and winter. Samples included parsley, coriander, basil, savory, mint, cress, tarragon, leek, radish and, spring onion which

© 2018 The Author(s); Published by Hamadan University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
were purchased from different farms or markets.

Sample Preparation and Parasite Detection
A total of 380 vegetable samples were collected from urban and rural areas of Hamadan. Two hundred to 250 g of each vegetables was collected separately in zipped bags. Soil and coarse particles were removed from the samples before they were transferred to Parasitology Research Laboratory of Hamadan University of Medical Sciences. Procedures were followed according to those used in previous studies (4,6-9). Briefly, each weighed sample was left in 1 L of tap water containing 2% of detergent for 1 hour during which the samples were shaken constantly. The vegetable was picked up slowly and the remained water was left for 12 hours. Then the supernatant liquid was discarded and the sediment was passed through the sieve no. 40 (425 µm) to eliminate large particles and to monitor the larva or free living nematodes. The sample at the final volume of 50 mL was centrifuged at 500 rpm for 15 minutes, a drop of sediment was placed on a clean glass slide under microscopy for detection of eggs, larva or protozoan cysts (10). The zinc sulfate flotation tests were also done. Protozoan cysts were identified using Lugol’s iodine following Trichrome staining technique for accurate differentiation (9).

Data Analysis
Statistical analysis was carried out using chi-square test by the SPSS software version 24. Potential differences in contamination among different seasons, samples and irrigation systems were considered significant at $P<0.05$.

Results
Parasites were detected in 95 out of 380 samples. Twenty-five percent of samples were found to be contaminated. The most prevalent parasite was *Entamoeba coli* that was detected in 8.15% of samples, followed by *Ascaris lumbricoides* and *Chilomastix mesnili*. The highest rate of contamination was reported in parsley (5.52%) and the lowest in radish (0.78%). Distribution of parasites based on types of vegetables is listed in Table 1.

Based on seasonal variation, parasitic contamination rate was highest in spring (8.67%) and lowest in winter (0%). Vegetables showed more contamination in spring and summer than in autumn and winter ($P<0.0001$). The results showed that 14.47% of protozoan cysts and 1.8% of worm eggs were identified in vegetables collected in spring and summer. The contamination results showed 3.9% of protozoan cysts and 3.6% of egg worms in autumn and winter.

Regarding irrigation procedures in farms, 31.5% of vegetables were cultivated in farms irrigated by surface or sewage waters and 55.2% of vegetables in farms irrigated by well water supply. The analysis revealed that in 11 of 12 farms (91%) irrigated by surface or sewage water sources, vegetables with at least one parasitic contamination had been produced whereas 6 of 21 (28.5%) the products cultivated in farms irrigated by well water supply were contaminated with at least one parasite. Samples collected from farms irrigated by well water supply showed the lowest contamination compared to those from farms irrigated by surface or sewage water ($P<0.0001$).

Discussion
The consumption of fresh fruits and vegetables is considered as a determinant of healthy diet. Despite the advantages of raw vegetables, they are regarded as a potential source of microbial transmission to human (7). Although dietitians advise eating raw vegetables in order to remain healthy, the hygienic way of using them is highlighted more than ever (11). Isolation of parasites from vegetables is characteristic for all processes involved in planting, irrigation, growing and harvesting (12,13). In the present study, total parasite

| Parasites          | Samples Tested No. | Ascaris lumbricoides Ova, No. (%) | Toxocara Ova, No. (%) | Hymenolepis nana Ova, No. (%) | Entamoeba Cyst, No. (%) | Giardia lamblia Cyst, No. (%) | Chilomastix mesnili Cyst, No. (%) | Iodamoeba butschlii Cyst, No. (%) | Blastocystis hominis Cyst, No. (%) | Total |
|--------------------|--------------------|-----------------------------------|-----------------------|------------------------------|------------------------|-----------------------------|----------------------------------|----------------------------------|---------------------------------|-------|
| Parsley            | 38                 | 2 (0.52)                          | -                     | -                            | 6 (1.57)               | -                           | 8 (2.1)                          | 3 (0.78)                         | 2 (0.52)                         | 21    |
| Coriander          | 38                 | 2 (0.52)                          | -                     | -                            | 2 (0.52)               | 4 (1)                       | -                                | -                                | -                              | 8     |
| Basil              | 38                 | 1 (0.26)                          | -                     | -                            | 1 (0.26)               | -                           | 2 (0.52)                         | -                                | -                              | 4     |
| Savory             | 38                 | 3 (0.78)                          | -                     | -                            | 2 (0.52)               | -                           | 4 (1)                            | 1 (0.26)                         | -                              | 10    |
| Mint               | 38                 | 1 (0.26)                          | 1 (0.26)              | -                            | 7 (1.84)               | 2 (0.52)                    | 3 (0.78)                         | 1 (0.26)                         | -                              | 15    |
| Cress              | 38                 | 4 (1)                             | -                     | -                            | 3 (0.78)               | 4 (1)                       | -                                | -                                | -                              | 11    |
| Tarragon           | 38                 | 4 (1)                             | -                     | -                            | 4 (1)                  | 1 (0.26)                    | -                                | -                                | 1 (0.26)                        | 9     |
| Leek               | 38                 | 3 (0.78)                          | -                     | -                            | 6 (1.57)               | -                           | -                                | -                                | -                              | 10    |
| Radish             | 38                 | 3 (0.78)                          | -                     | -                            | -                      | -                           | -                                | 1 (0.26)                         | -                              | 3     |
| Spring onion       | 38                 | -                                 | 1 (0.26)              | 2 (0.52)                     | -                      | 1 (0.26)                    | -                                | -                                | -                              | 4     |
| Total              | 380                | 23 (6)                            | 1 (0.26)              | 1 (0.26)                     | 33 (8.68)              | 6 (1.57)                    | 23 (6)                           | 4 (1)                            | 3 (0.78)                        | 95    |
contamination of vegetables was estimated at 25%. This result is consistent with studies of Alhabbal in Syria (31.38%) (13), Fallah et al in Shahrekord, southwest Iran (32.6%) (14) and Ishaku et al in Nigeria (20%) (15). In recent studies conducted near Hamadan, comparatively lower contamination rates have been recorded; for example, Rahmati et al (9) and Matini et al (6) reported that 14.6% and 8.4% of raw vegetables were contaminated, respectively. Weather condition, irrigation system, type of fertilizer, the sewage system and the rate of regional parasitic infection are effective on the rates of contamination in these areas. In this study, *E. coli* was found as the most predominant parasite in all samples (2.6%). This result is in agreement with the finding of Matini et al that reported *E. coli* as the most frequently detected parasite with the prevalence of 8.15% (6). In the study of Gabre and Shakir (16) in Saudi Arabia, *E. coli* was found as the most frequent parasite (23.9%) and Daryani et al (17) reported that 10% of vegetables in Ardabil were contaminated with *E. coli*. Although *E. coli* is not regarded as a parasite, the existence of this microorganism in fruits or vegetables is an indicator of human feces influx into fresh vegetables (8,17,18).

*Ascaris lumbricoides* was the second leading common parasite found in vegetables (7.1%). Studies conducted in Shahrekord, Iran (14), India (19) and Ethiopia (20) showed the prevalence of *A. lumbricoides* were 14.1%, 36% and 20.83%, respectively. *A. lumbricoides* is one of the most important soil transmitted parasites whose prevalence has recently been reported to range from 1.5% to 53.2% in the world (13). Though the incidence of ascariasis has decreased in recent years, vegetable contamination by this parasite may indicate the potential risk of its re-emergence in the region (6). Contamination of vegetables by the helminth eggs might be associated with reusing raw wastewater in irrigation, using human fertilizers and post-harvest handling. In addition, the helminth eggs including *Ascaris ova* are very resistant to environmental factors and are transmitted to consumers easily (19).

In the current study, parsley was the most common contaminated vegetable (5.52%). Alhabbal (13) in Syria and Gharei et al (21) in Iran observed the predominance of contaminated parsley in the vegetables. This could be attributed to dense foliage, rough surface and deep grooves of this plant, protecting protozoa cysts and helminth eggs against environmental threats.

Regarding seasonal variability, contamination of vegetable samples was highest in spring (8.6%) and summer (7.6%) and lowest in autumn (6.7%) and winter (0) (*P* < 0.005). Previous studies have confirmed the higher parasitic contamination and parasitic diversity of vegetables during warm seasons (14,22,23). In Hamadan, water supplies are provided by the river water, untreated waste water or surface water to be used for irrigation of vegetables cultivated throughout spring and summer, while during autumn and winter, vegetables are imported mostly from the farms of Khuzestan, southwest of Iran, which are irrigated by rain or underground water. Furthermore, increased number of parasites in vegetables during warm seasons might be due to the increase of the temperature intensifying the excretion of parasites by humans or animals (24).

Surface water can be contaminated with human or animal feces in the path and the resulting contamination may transmit the helminth ova or protozoan cysts to vegetables. Bekele found out the determinant role of water supply in contamination of vegetables (20). In our study, 31.5% of vegetable samples had been cultivated in farms irrigated by surface or sewage water, while 55.2% of vegetables were obtained from farms irrigated by well water. Our investigations showed that 28.5% and 91% of products irrigated by, respectively, well water and surface water were contaminated with at least one parasite species. A significant difference was observed between parasitic contamination and irrigation system (*P* < 0.005).

Conclusions
The result of present study indicated that raw vegetables could be a potential source of parasitic infection. Regarding local preference for raw vegetables, the way of proper washing of vegetables should be educated in order to avoid the adverse health effects of intestinal parasites. It is necessary to improve the knowledge of local farmers about disadvantages of using surface or waste water for irrigation of vegetables.

Ethical Approval
The present study was approved by Ethics Committee of Hamadan University of Medical Sciences (Ethics Committee reference number: IR.UMSHA.REC.1396.131).

Conflict of Interest Disclosures
The authors declare that they have no conflict of interests.

Acknowledgements
The authors would like to express their gratitude and appreciation to the Student Research Center of Hamadan University of Medical sciences for their financial support (Grant number: 9602261180).

References
1. Liu RH. Health-promoting components of fruits and vegetables in the diet. Adv Nutr. 2013;4(3):384s-92s. doi: 10.3945/
an.112.003517.
2. 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.
3. Jung Y, Jang H, Matthews KR. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microb Biotechnol. 2014;7(6):517-27. doi: 10.1111/1751-7915.12178.
4. Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, et al. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. Int J Food Microbiol. 2010;139 Suppl 1:S3-15. doi: 10.1016/j.
Parasitic contamination of vegetables in Hamadan

5. Iran Statistics Center. Implementation of the 2016 Iranian population and housing census in autumn 2016. 2016.

6. Matini M, Shamsi-Ehsan T, Maghsod AH. The Parasitic Contamination of Farm Vegetables in Asadabad City, West of Iran, in 2014. Avicenna J Clin Microbiol Infect. 2017;4(1):e32474-. doi: 10.17795/ajcmi-32474.

7. Menezes MC, Costa BV, Oliveira CD, Lopes AC. Local food environment and fruit and vegetable consumption: An ecological study. Prev Med Rep. 2017;5:13-20. doi: 10.1016/j.pmedr.2016.10.015.

8. Mohamed MA, Siddig EE, Elaagip AH, Edris AM, Nasr AA. Parasitic contamination of fresh vegetables sold at central markets in Khartoum state, Sudan. Ann Clin Microbiol Antimicrob. 2016;15:17. doi: 10.1186/s12941-016-0133-5.

9. Rahmati K, Fallah M, Maghsod AH, Shamsi-Ehsan T, Matini M. The Prevalence of Parasitic Contamination of Vegetables Consumed in Malayer City, West of Iran, in 2014. Avicenna J Clin Microbiol Infect. 2017;4(2):e42380-. doi: 10.5812/ajcmi-42380.

10. Garcia LS. Macroscopic and microscopic examination of fecal specimens. In: Diagnostic medical parasitology. 5th ed. Washington, DC: ASM Press; 1993:501-35. doi: 10.1128/9781555816018.ch27.

11. Jay-Russell MT. What is the risk from wild animals in food-borne pathogen contaminated plants. CAB Rev. 2013;8(40):1-16.

12. Alade GO, Alade TO, Adewuyi IK. Prevalence of intestinal parasites in vegetables sold in Ilorin, Nigeria. Am Eurasian J Agric Environ Sci. 2013;13(9):1275-82. doi: 10.5829/ideosi.aejs.2013.13.09.11040.

13. Alhabbal AT. The prevalence of parasitic contamination on common cold vegetables in Alqalamoun Region. Int J Pharm Sci Res. 2015;30(1):94-7.

14. 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.

15. Akyala Ishaku A, Ihekuku D, Agwale S. Prevalence of parasitic contamination of some edible vegetables sold at alhamis market in Ijebu Metropolis. Scholarly Journal of Biotechnology. 2013;2(2):26-9.

16. Gabre RM, Shokir A. Prevalence of some human enteroparasites in commonly consumed raw vegetables in Tabuk, Saudi Arabia. J Food Prot. 2016;79(4):655-6. doi: 10.4315/0362-028x.jfp-15-485.

17. Daryani A, Ettehad GH, Shariﬁ 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.

18. Balarak D, Modrek MJ, Bazrafshan E, Ansari H, Kord Mostafapour F. Prevalence of intestinal parasitic infection among food handlers in northwest Iran. J Parasitol Res. 2016;2016:8461965. doi: 10.1155/2016/8461965.

19. Gupta N, Khan DK, Santra SC. Prevalence of intestinal helmynth eggs on vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. Food Control. 2009;20(10):942-5. doi: 10.1016/j.foodcont.2009.02.003.

20. Bekele F, Tefera T, Biresaw G, Yohannes T. Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. Infect Dis Poverty. 2017;6(1):19. doi: 10.1186/s40249-016-0226-6.

21. Gharavi MJ, Jahani MR, Rokni MB. Parasitic contamination of vegetables from farms and markets in Tehran. Iran J Public Health. 2002;31(3-4):83-6.

22. Al-Megrin WA. Prevalence of 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.

23. Eraky MA, Rashed SM, Nasr Mel S, El-Hamshary AM, Salah El-Ghannam A. Parasitic contamination of commonly consumed fresh leafy vegetables in benha, egypt. J Parasitol Res. 2014;2014:613960. doi: 10.1155/2014/613960.

24. Yusof AM, Mohammad M, Abdullahi MA, Mohamed Z, Zakaria R, Wahab RA. Occurrence of intestinal parasitic contamination in select consumed local raw vegetables and fruits in Kuantan, Pahang. Trop Life Sci Res. 2017;28(1):23-32. doi: 10.21315/tlshr2017.28.1.2.