Cryptosporidium oocyst and *Giardia* cyst contamination of salad vegetables in Kufra city, Libya

Frhat M. A. Saaed[1], Jerry E. Ongerth[2]
[1]Department of Zoology, College of Arts and Sciences, Benghazi University, Kufra, Libya
[2]Principal Honorary Fellow, Civil, Mining, & Environmental Engineering, University of Wollongong, Wollongong, Australia
Corresponding author, E-mail addresses: fmasm72@yahoo.com.au (F. M. Saaed)

ABSTRACT

Background: Raw (fresh) vegetables as part of a healthy diet is an important key to help boost immune health. However, they could also have a negative impact on humans. Vegetables, especially those that are consumed raw and not properly washed, have been the major way for the transmission of human pathogens. For example, *Cryptosporidium* and *Giardia* infection may be acquired in different ways including by consumption of contaminated vegetables and water. The purpose of this paper is to describe the detection of *Giardia* cyst and *Cryptosporidium* oocyst contamination in some common salad vegetables sold at the small retail and local markets in Kufra city, Libya.

Methods: A total of 250 samples including five different types of fresh salad vegetables were randomly collected and examined between July 2013 and February 2014, for the prevalence of both parasites by using local accepted standard method, similar to USEPA Method 1623, followed by concentration of target organisms from other particulates by IMS then completed by IFA and microscopy as specified by Method 1623.

Results: It was found that, in the 250 samples examined, 211 (84.4%) were contaminated with *Giardia* cysts and 202 (80.8%) were contaminated with *Cryptosporidium* oocysts. Of the 50 lettuce, 50 parsley, 50 Watercress (*Eruca Sativa*), 50 tomato, and 50 cucumber samples examined cysts of *Giardia* spp. were detected in 96%, 94%, 96%, 64% and 72%, respectively and oocysts of *Cryptosporidium* spp. were detected in 96%, 86%, 90%, 60% and 72%, respectively. Our results indicate that the parasitic contamination of salad vegetables found in our study is likely caused by irrigating crops with faecal contaminated water.

Conclusion: *Giardia* cysts and *Cryptosporidium* oocysts contamination of raw salad vegetables sold in Kufra city may cause a health risk to consumers of such products.

Key words: Cryptosporidium, Giardia, contamination, salad vegetable, Kufra-Libya

Introduction

Intestinal parasitic infections are widely distributed throughout the world causing substantial intimidation to the public health, economy, and physical and cognitive development particularly among children in developing countries, (20, 28). The prevalence of parasitological contamination is more common in the areas where the sanitation, personal hygiene, environmental hygiene, and health system commonly are poor and the places in which the raw human faeces are used as fertilizer (32, 49, 53). Transmission is via the faecal-oral route through the ingestion of oocysts in contaminated food or water, direct contact with animals, or person-person contact (12, 29). *Cryptosporidium* oocysts and *Giardia* cysts are of public health significance requiring monitoring of their presence in drinking water (52). Previous studies have reported that *Cryptosporidium* and *Giardia* in surface water used for public water supply range in concentration from 0.01 organisms/L to as many as 10 organisms/L (33, 35). Use of quality control tools such as carefully seeded positive controls is an essential part of any method for this type of monitoring (33, 34). This is particularly important recognizing that the most frequent result of analysing 10 L surface water samples is zero (33). The most widely used method for monitoring *Cryptosporidium* and *Giardia* is the USEPA Method 1623 (50). The method stipulates QA procedures requiring measurement of the number of organisms recovered from at least 10 L sample volumes following addition of 100 to 500 cysts and oocysts.

Both organisms are common water contaminants and have been detected in irrigation water, effluents, and biosolids from wastewater treatment plants (13, 14, 24, 31, 39). *Cryptosporidium* and *Giardia* have been detected on vegetables and in water samples from field irrigation (6, 24). Fresh produce, in particular, as it is consumed with minimal preparation, is a potential vehicle of transmission, and *G. duodenalis* cysts have been detected on produce in several countries (3, 41, 42, 43). Contaminated irrigation water, especially, appears to constitute a major route of contamination of fresh produce (15, 17, 49). Raw (fresh**)** vegetables can be agents of transmission of *Cryptosporidium* oocysts and *Giardia* cysts (1, 2, 24), and outbreaks of intestinal parasitic infections epidemiologically associated with raw vegetables have been reported from developed and developing countries (2, 30, 36). Information on *Giardia* cysts in fresh vegetables sold in Libya (Tripoli city) have been reported (2). Many studies have been conducted to evaluate the role of raw vegetables in the transmission of intestinal parasites, for example, in Alexandria, Egypt; Tripoli, Libya; Riyadh, Saudi Arabia; Iraq; Tehran and Qazvin Province, Iran; and the Philippines (28). All have stressed the importance of fruits and vegetables, particularly which are consumed raw and unwashed, in the transmission of medically important parasites (10). Fresh vegetables are clearly an effective transmission vehicle if subject to faecal contamination since many protozoan cysts, helminthes eggs and larvae can be present and thus consumed directly in the contaminated vegetables (1, 16, 18). Studies have shown that *Ascaris lumbricoides*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Enterobius vermicularis*, *Fasciola* spp., *Giardia intestinalis*, *hookworm*, *Hymenolepis* spp., *Taenia* spp., *Trichuris trichiura*, and *Toxocara* spp., can infect humans consuming contaminated, uncooked, or improperly washed vegetables and fruits (10, 27). We used the method below to detect oocysts and cysts on a variety of Fresh vegetables such as lettuce, parsley, Watercress (*Eruca Sativa*), tomato, and cucumber obtained from local markets in the Kufra city. These vegetables were chosen because they are among the major components of the daily diet in Libya, in which vegetable salads are a daily component. The level of parasitological contamination of fruits and vegetables still need to be estimated. To the best of our knowledge, few published documents on freshly eaten vegetables in Libya and few previous surveys have been conducted to evaluate the parasitic contamination of fresh vegetables only in Tripoli, the capital city. Therefore, this study was conducted to determine the level of parasitic contamination of selected fresh vegetables such as lettuce, parsley, watercress (*Eruca Sativa*), tomato, and cucumber that are frequently eaten raw were obtained from local markets in the Kufra city.

Materials and methods

The study was conducted in Kufra city in southeast Libya, about 1700 km by road from Tripoli, the capital city. A total of 250 samples of five different types of fresh salad vegetables were taken from smallest retail size available and local markets of Kufra city between July 2013 and February 2014. Vegetable supplies of shops in the city were identified and then 500 g of samples were taken from each of them under normal purchase conditions. Samples were collected from the upper, middle and lower shelves of each seller. These vegetables were collected into sterile, labeled plastic bags and transported to the Laboratory within 1 h of collection for the examination and were studied on the same day. The leaves of each sample were completely separated using disposable and clean gloves. Samples of each vegetable (100 g) were washed (soaked for 15 minutes, followed by vigorous shaking for 15 minutes) separately in clean and sterile glass bottles., which contained three liters of particle-free filtered-Milli-Q water for removing the parasitic stages (cysts, and oocysts) of protozoan parasites commonly assumed to be associated with vegetable contamination. The filter bag containing the vegetable sample was removed and then discarded. The wash water sample was then processed and examined for the presence of oocysts and cysts of *Cryptosporidium* spp. and *Giardia* spp.

The approach used in this paper is a locally accepted standard method, similar to USEPA Method 1623, consisting of 2 µm pore dia. 293 mm dia flat polycarbonate membrane filtration for collection of *Cryptosporidium* oocysts and *Giardia* cysts together, followed by concentration of target organisms from other particulates by IMS, then completed by IFA and microscopy as specified by Method 1623. Rigorous negative controls were included to demonstrate lack of contamination, (21).

The three liter wash water samples were filtered using a 2 µm pore dia. 293 mm dia flat polycarbonate membrane mounted in a stainless steel filter holder. Water was drawn through the filter under vacuum. After filtration, the filter was removed from the holder and placed face-up on pane of glass that was rested in an unused plastic collection pan. The filter was wetted from top to bottom with particle free filtered-Milli-Q water, using a wash bottle and squeegeed three times using a clean rubber (windscreen wiper blade section super-glued to a disposable razor body). The collected filter washing suspension was transferred to 50 ml disposable centrifuge tube then spun to pellet at 2500 rpm for 15 minutes. Supernatant was aspirated to 5 ml over the pellet. The resulting pellets were completely resuspended. Cysts and oocysts were separated from debris by Dynal Crypto-Combo immunomagnetic beads and Dynal *Giardia*-Combo immunomagnetic beads (IMS). The sample on the well slide was allowed to air dry completely before staining for immunofluorescence assay, including DAPI stain to provide for visualization of nuclei as an aide to identification. Negative controls samples consisting of 3L of particle-free (filtered) MilliQ water were processed by the full method to ensure that no cysts and oocysts were introduced or carried over into water sample being analyzed. They were run prior to the first time these experiments started and before processing each sample to ensure that no oo/cysts were carried over between samples. *Cryptosporidium* oocysts and *Giardia* cysts were identified based on the comparison with the fluorescent features of the (oo)cysts and followed the described criteria (U.S. Environmental Protection Agency 2005). The confirmation of (oo)cysts was also based on the results of DAPI staining, and differential interference contrast microcopy. The entire well was examined, an up-and- down or a side-to- side for bright apple-green fluorescence of the (oo)cysts wall, and shape using epifluorescence microcopy.

Results

In the study of five different types of fresh salad vegetables (50 lettuce, 50 parsley, 50 watercress (*Eruca sativa*), 50 tomato, and 50 cucumber), we observed oocysts of *Cryptosporidium* in (202 out of 250) samples (80.8%) and cysts of *Giardia* in (211 out of 250) samples (84.4%), Table 1. The highest percentage of positive samples was observed for both *Cryptosporidium* (96%) and *Giardia* (96%) in Lettuce, but only slightly less in samples of watercress (90%, and 96%, respectively) and in samples of parsley (86%, and 94%, respectively). Among the smooth vegetables samples, cucumber showed lower contamination percentages (72% for both Cryptosporidium and Giardia). The least percentage of contamination was found in tomato samples (60% and 64%, respectively).

Table 1: Presence of *Cryptosporidium* oocysts and *Giardi*a cysts measured in samples according to vegetable type.

Type of produce Sample (100 g)	Number examined	Number positive *Cryptosporidium* (%)	Number positive *Giardia* (%)
Lettuce	50	48 (96)	48 (96)
Parsley	50	43 (86)	47 (94)
Watercress	50	45 (90)	48 (96)
Tomato	50	30 (60)	32 (64)
Cucumber	50	36 (72)	36 (72)
Total	250	202 (80)	211 (84)

In almost all positive samples both *Cryptosporidium* and *Giardia* were found together, Table 2. In fact *Cryptosporidium* contamination was not found alone in any of the positive samples whereas *Giardia* contamination alone was found in only 9 of 202 positive samples. In general the leafy vegetables (lettuce, parsley, and watercress) averaged nearly 90% positive for both *Cryptosporidium* and for *Giardia* whereas about 1/3rd of the tomato and cucumber samples were found with no oocyst or cyst contamination. Overall, the most common parasite found in the five different types of fresh salad vegetables samples was *Giardia* cysts but only by a narrow margin. Overall, these representative commercially purchased vegetables eaten raw were almost uniformly found contaminated with the two protozoan parasites.

Table 2 General information, *Cryptosporidium* oocyst and *Giardia* cyst contamination levels in five different types of fresh salad vegetables in the city of Kufra, Libya.

Samples (100 g)	No. of samples with only oocysts	No. of samples with only cysts	No. of samples with both oocysts & cysts	No. of samples of with no oocysts	No. of samples with no cysts	Total
Lettuce	0	0	48	2	2	50
Parsley	0	4	43	7	3	50
Watercress	0	3	45	5	2	50
Tomato	0	2	30	20	18	50
Cucumber	0	0	36	14	14	50
Total	0	9	202	48	39	50

Discussion

Cryptosporidium oocysts and *Giardia* cysts have a wide distribution and consistent presence among human and animals, shed in the faeces of infected hosts. Vegetables are one of the major components of the daily diet in Libya. Grown and marketed locally salad vegetables (cucumber, tomato, lettuce, watercress (*Eruca sativa*) and parsley) are one of most common the daily dishes in the Libyan diet. As illustrated by the findings summarized above, these vegetables that are eaten raw can be a major source of parasitic infection. The results suggest that an important route of parasitic infection in humans, especially in developing countries is the consumption of raw vegetables. Raw vegetables may become contaminated by faecal-origin pathogens while growing in the field through irrigation such as the type of water used including sewage origin or contaminated water, soil or types of fertilizers used (sewage sludge, animal manure and compost). Other potential routes of contamination such as, hygienic conditions, faecal waste from different animals belonging to farmers, and fertilization procedures. Climate and season may contribute to contamination of the edible part of leafy vegetable with accretion of contaminated soil. Some organic farming practices, and details of harvesting, handling, transport, and storage of the vegetables may also have the potential to confer contamination (1,11, 22).

Our study revealed the presence of *Cryptosporidium* oocysts and *Giardia* cysts in the analyzed samples with an average (80.8% and 84.4%, respectively). This percentage was the highest report among the other previous studies from different countries, in Valencia, Spain, the occurrence of *Cryptosporidium* oocysts and *Giardia* cysts on vegetables reported (63.1% and 52.6%), respectively (24), also in Ilam city, Iran reported (30.7% and 6.8%) for *Cryptosporidium* oocysts and *Giardia* cysts, respectively, the overall contamination reported 37% (8), in Alexandria- Egypt, the presence of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts reported (29.3% and 6.7%), respectively (45), In Shiraz city, reported (23.5%) with *Cryptosporidium* contamination (40), vegetables in Nitro in Brazil, were reported (26%) with *Cryptosporidium* infection (38), in Ahvaz, south west of Iran reported 15.5% with *Giardia* spp. (46), in Jiruft citiy, Iran reported 14% with *Giardia* spp. (7, 54), in Jimma town, Ethiopia, contamination percentage reported (12.8% and 7.5%)**,** respectively (48), in Yasouj city, Iran reported 11% with *Giardia* spp. (47), in Shiraz, reported 10% with *Giardia* cysts (7), in Tripoli, Libya reported 10% with *Giardia* cysts (2), in Amman and Baqa'a, Jordan, reported (9%) with cysts of *Giardia* spp. (25), in Arba Minch town, Southern Ethiopia, the prevalence and predictors of *Cryptosporidium* spp.; and cysts of *G. intestinalis* contamination of fruits and vegetables reported (4.72% and 10.0%), respectively (10), in Ardabil citiy, Iran reported 8.9% with *Giardia* spp. (7, 18), in Norway from 1999 to 2001 reported (6%) with *Giardia* cysts (44), in Norway, reported (6%) with *Cryptosporidium* infection (37), in Shahrekord citiy, Iran reported 5% with *Giardia* spp. (23), in Ramhormuz, reported (4.6%) with *Giardia* (5), in Isfahan city, reported Seven types of Giardia in 480 samples of edible vegetables (26), In Norway and Costa Rica, reported (2% and 5%, respectively) with *Giardia* (19, 7), in Dhamar city, Yemen contamination percentage of *Giardia lamblia* reported (3.8%) (1). Lower rates of contamination were reported in Shiraz, southwest of Iran, (3.7% and 4.8%) with *Giardia* cysts and (1.2% and 1.3%) with *Cryptosporidium* spp. In total 31.2% (25/80) and 36.8% (53/144) of retails and farms vegetables, respectively (7).

In the samples analyzed here higher percentages of positive samples were observed for both *Cryptosporidium* and *Giardia* in five different types of fresh salad vegetables. *Cryptosporidium* spp. was detected in this study with a prevalence of

80.8%. This finding is higher than the finding of previous studies. Nonetheless, majority of researches did not report the parasite as vegetable contaminant (49). This might be due to differences in methods used between this study and the previous ones. Standard methods for detecting protozoan parasites on salad vegetables are not widely available or standardized. Published techniques for the isolation of parasites from vegetables generally have low and variable recovery efficiencies (24). The difference between the current study and previous studies may be as a result of the kind of sample and sample size examined, the sampling techniques, methods used for detection *Cryptosporidium* oocysts and *Giardia* cysts. Use of our rigorous and straightforward procedures are likely more efficient than used in the majority of the previous studies. So long as these factors differ, difference of results must be expected.

Lettuce (96% for oocysts and cysts) was found to be the most frequently contaminated produce followed by watercress (*Eruca sativa*) (90% and 96% for oocysts and cysts, respectively), and parsley (86% and 94% for oocysts and cysts, respectively). Cucumber (72.2% for oocysts and cysts), and tomato (60% and 64% for oocysts and cysts, respectively) were the least contaminated. This variation among types of produce may be due to their broad leaves that provide large contact area with contaminated irrigation water and soil (48). Lettuce, watercress (*Eruca Sativa*), and parsley have uneven surfaces that foster accumulation of soil contaminated with parasitic stages to attach more easily to the surface of these vegetables. Contamination may also occur and contaminants adhere when the leafy vegetables are washed with contaminated water either in the farm or market (9, 4, 27, 48). Vegetables with smooth surface as tomato and cucumber may limit the rate of parasitic attachment contributing to lower observed presence (45).

Conclusion

In conclusion, the data presented here illustrate the effectiveness of the local accepted standard method, similar to USEPA Method 1623, followed by concentration of target organisms from other particulates by IMS then completed by IFA and microscopy as specified by Method 1623 for detecting the presence of *Giardia* and *Cryptosporidium* in water samples from washing fresh vegetable samples. In this study the levels of *Cryptosporidium* infection were marginally less than *Giardia*. The presence of both parasites in a high percentage was detected. This appears to be a result of contamination from cultivation including watering, fertilizing, and harvesting procedures in generally contaminated environments. Ample opportunities for contamination are present during handling, transport and storage, or direct contamination from individuals involved in the production and processing of these vegetable products. The high prevalence of *Cryptosporidium* oocysts and *Giardia* cysts in fresj vegetables purchased and available for every-day consumption in Kufra city suggests that fresh produce eaten raw should be considered a significant source leading to parasitic infections among the community. More research is needed to evaluate the level of parasitic contamination in fresh vegetables such as (*Ascaris lumbricoides* eggs, *Toxocara* spp. eggs, *Taenia* spp. eggs, *Trichostrongylus* eggs, *Trichuris* eggs, *Hymenolepis nana* eggs, *Cyclospora* spp. oocysts and *Microsporidium* spp.) in the smaller rural cities such as Kufra, Libya. Such studies should also be conducted in different regions of the country to determine the extent of such high levels of faecal origin fresh vegetable contamination. Our study showed that raw vegetables sold in city of Kufra are contaminated with *Cryptosporidium* oocysts and *Giardia* cysts that may pose a health risk to consumers. Public health programs are needed to inform the public of the importance of effective food preparation in homes and restaurants comprehensive health education should also be given to vendors and farmers. Vendors should ensure that produce does not make contact with the soil when displaying for selling. Local health and environmental authorities should devote appropriate attention to improve the hygienic conditions in the areas where the salad vegetables are cultivated and consumed. *Giardia* cysts and *Cryptosporidium* oocysts contamination of raw salad vegetables sold in Kufra city are currently a likely cause of health risk to consumers of such products.

References

[1] Abdul-Wahab, A., Fahd, M. A., Bakeel, A. R., and Rassam, T. A. Prevalence of intestinal parasites in fresh leafy vegetables in some farms at Dhamar city, Yemen. International Journal of Medicine Research, Volume 1; Issue 5; November 2016; Page No. 07-13

[2] Abougrain, A. K, Nahaisi, M. H, Madi, N. S, Saied, M. M, Ghenghesh, K. S. Parasitological contamination in salad vegetables in Tripoli-Libya. *Food Control* 2010; **21**(5): 760-2.

[3] Amahmid, O., Asmama, S., & Bouhoum, K. (1999). The effect of waste water reuse in irrigation on contamination of food crops by Giardia cysts and Ascaris eggs. *International Journal of Food Microbiology*, 49, 19–26.

[4] Amoah, P., Drechsel, P. and Abaidoo, R. C., 2006. Pesticide and pathogen contamination of vegetables in Ghana's urban markets. *Arch. environ. Contam. Toxicol*., **50**: 1-6.

[5] Arab, L, Rahdar, M. Parasitic contamination of consumed vegetables from farms in Ramhormoz. *Jundishapur Scientific Medical Journal* 2006; 11(4): 375-82.

[6] Armon, R., D. Gold, M. Brodsky, and G. Oron. 2002. Surface and subsurface irrigation with effluents of different qualities and presence of *Cryptosporidium* oocysts in soil and on crops. Water Sci. Technol. 46:115–122.

[7] Asadpour, M., Hosein, M., Arash, J., and Somayeh, B. Diversity of parasitic contamination in raw vegetables commonly consumed in Shiraz, southwest of Iran. *Asian Pac J Trop Dis* 2016; 6(2): 160-162

[8] Avazpoor, M, Yousefipoor, M. T., Dusty, M, Mehdipour, M, Seifipour, F, Gholami, Z. Determination of the level of parasitic infection (Cryptosporidium and Giardia) of the vegetables marketed in Ilam city. *Environmental Health Engineering and Management Journal* 2015; 2(1): 37–40.

[9] Avcioglu, H., Soykan, E. and Tarakci, U., 2011.Control of helminth contamination of raw vegetables by washing. *Vector Borne Zoonot. Dis.*, **11**:189-91.

[10] Bekele, F., Tamirat, T., Gelila, B., and Tsegaye, Y. Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. Infectious Diseases of Poverty (2017) 6:19

[11] Bouhoum, K, Amahmid, O. Municipal wastewater reuse for irrigation: Productivity and contamination level of irrigated crops by pathogens. In Proceedings of International Symposium on Environmental Pollution Control and Waste Management. 2002; 7:7-10.

[12] Casemore, D. (1990) Epidemiological aspects of human cryptosporidiosis. Epidemiol Infect. 104, 1–28

[13] Caccio`, S. M., M. de Giacomo, F. A. Aulicino, and E. Pozio. 2003. *Giardia* cysts in wastewater treatment plants in Italy. Appl. Environ. Microbiol. 69:3393–3398.

[14] Castro-Hermida, J. A., I. Garcı´a-Presedo, A. Almeida, M. Gonza´lez- Warleta, J. M. Correia Da Costa, and M. Mezo. 2008. Contribution of treated wastewater to the

contamination of recreational river areas with *Cryptosporidium* spp. and *Giardia duodenalis*. Water Res. 42: 3528–3538.

[15] Chaidez, C., M. Soto, P. Gortares, and K. Mena. 2005. Occurrence of *Cryptosporidium* and *Giardia* in irrigation water and its impact on the fresh produce industry. Int. J. Environ. Health Res. 15:339–345.

[16] Coelho, L. M, Oliveira, S. M, Milman, M. H, Karasawa, K. A, Santos, R. D. Detection of transmissible forms of enteroparasites in water and vegetables consumed at schools in Sorocaba, Sao Paulo state, Brazil. Rev. Soc. Bras. Med. Trop. 2000; 34:479-82.

[17] Cook, N., R. A. B. Nichols, N. Wilkinson, C. A. Paton, K. Barker, and H. V. Smith. 2007. Development of a method for detection of Giardia duodenalis cysts on lettuce and for simultaneous analysis of salad products for the presence of *Giardia* cysts and *Cryptosporidium* oocysts. Appl. Environ. Microbiol. 73:7388–7391.

[18] Daryani, A., Ettehad, G., Sharif, M., Ghorbani, L., Ziaei, H. Prevalence of intestinal parasites in vegetables consumed in Ardabil, Iran. Food control. 2008; 19:790-794.

[19] Davami, M, Mosayyebi, M, Mahdavipour, A. Prevalence of parasitic infections in consumed vegetables in Ardabil city. *J Rah Avarde Danesh* 2000; **3**: 18-22.

[20] Efstratiou, A, Ongerth, JE, Karanis, P., 2017a. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks-An update 2011-2016. Water Research. 110:1-9. http://dx.doi.org/10.1016/j.watres.2017.01.036

[21] Efstratiou, A, Ongerth, J, Karanis, P, 2017b. Evolution of Monitoring for *Giardia* and *Cryptosporidium* in Water. Water Research 123:96-112. http://dx.doi.org/10.1016/j.watres. 2017.06.042

[22] Ezatpour, B, Chegeni, A. S, Abdollahpour, F, Aazami, M, Alirezaei, M. Prevalence of parasitic contamination of raw vegetables in Khorramabad, Iran. Food control. 2013; 34:92-95.

[23] Fallah, A. A, Pirali-Kheirabadi, K, Shirvani, F, Saei-Dehkordi, S. S. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: influence of season and washing procedure. *Food Control* 2012; **25**: 617-20.

[24] Inmaculada, A., Jose, L. A., and Gonzalo, C. *Cryptosporidium* Oocysts and *Giardia* Cysts on Salad Products Irrigated with Contaminated Water. Journal of Food Protection, Vol. 73, No. 6, 2010, Pages 1138–1140.

[25] Ismail, Y. Prevalence of Parasitic Contamination in Salad Vegetables Collected from Supermarkets and Street Vendors in Amman and Baqa'a – Jordan. Polish Journal of Microbiology. 2016, Vol. 65, No 2, 201–207.

[26] Izadi, S, Abedi, S, Ahmadian, S, Mahmoodi, M. Study of the current parasitic contamination of the edible in Isfahan in order to identify preventive measures. Journal of Kurdistan University of Medical Sciences 2006; 11(2): 51-8.

[27] Kozan, E, Gonenc, B, Sarimehmetoglu, O, Aycicek, H. Prevalence of helminth eggs on raw vegetables used for salads. Food Control. 2005;16:239–42.

[28] Mahmoudi, M-R, Ongerth, J. E, Karanis, P. 2017. *Cryptosporidium* and cryptosporidiosis: The Asian perspective. International Journal of Hygiene and Environmental Health, 220(7):1098-1109. https://doi.org/10.1016/j.ijheh.2017 .06.005.

[29] McKerr, C, Adak, G. K, Nichols, G, Gorton, R, Chalmers, R. M, Kafatos, G, et al. (2015) An Outbreak of Cryptosporidium parvum across England & Scotland Associated with Consumption of Fresh Pre-Cut Salad Leaves, May 2012. PLoS ONE 10(5): e 0125955. doi:10.1371/journal.pone.0125955

[30] Mintz, E. D., Hudson-Wragg, M., Meshar, P., Carter, M. L., & Hadler, J. L. (1993). Foodborne giardiasis in a corporated office setting. Journal of Infectious Diseases, 167, 250–253.

[31] Montemayor, M., F. Valero, J. Jofre, and F. Lucena. 2005. Occurrence of *Cryptosporidium* spp. oocysts in raw and treated sewage and river water in north-eastern Spain. J. Appl. Microbiol. 99: 1455–1462.

[32] Okyay, P., Ertug, S., Gultekin, B., Onen, O., and Beser, E. "Intestinal parasites prevalence and related factors in school children, awestern city sample-Turkey," *BMC PublicHealth*, vol. 4, article 64, 2004.

[33] Ongerth, J. E., and Saaed, F. M. A., 2017. *Cryptosporidium* oocysts and *Giardia* cysts--A practical and sensitive method for counting and manipulating small numbers. Acta Tropica 169(5):119-121. http://dx.doi.org/10.1016/j.actatropica .2017.02.008.

[34] Ongerth, J. E., 2013. The concentration of *Cryptosporidium* and *Giardia* in water--the role and importance of recovery efficiency. Water Research, 47(7):2479-2488.

[35] Ongerth, J. E., 2017. *Cryptosporidium* and *Giardia* in Water: Reassessment of occurrence and significance. Am. Soc. Civil Engin., J. Environ. Eng., 2017, 143(3): 04016084, http://dx.doi.org/10.1061/(ASCE)EE.1943-7870.0001161)

[36] Ortega, Y. R., Roxas, C. R., Gilman, R. H., Miller, N. J., Cabrera, L., Taquiri, C., et al. (1997). Isolation of Cryptosporidium parvum and Cyclospora cayetanensis from vegetables collected in markets of an endemic region in Peru. American Journal of Tropical Medicine and Hygiene, 57, 683–686.

[37] Overgaauw, P. A, van Zutphen, L, Hoek, D, Yaya, F. O, Pinelli, E, et al. Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. Veterinary Parasitology 2009; 163 (1): 115-22.

[38] Paula, P, Rodrigues, P. S, Tortora, J. C, Uchoa, C. M, Farage, S. [Microbiological and parasitological contamination of lettuce (Lactuca sativa) from self service restaurants of Niterói city, RJ]. Rev Soc Brasil Med Trop 2003; 36(4): 535-7.

[39] Quintero-Betancourt, W., A. L. Gennacaro, T. M. Scott, and J. B. Rose. 2003. Assessment of methods for detection of infectious Cryptosporidium oocysts and Giardia cysts in reclaimed effluents. Appl. Environ. Microbiol. 69:5380–5388.

[40] Razavi, S, Nasirinasab-Rafsanjani, M, Bahrami, S. A study on *cryptosporidium* contamination in lettuce collected from different areas in Shiraz. Journal of Shahrekord University of Medical Sciences 2009; 12(2): 44-50.

[41] Robertson, L. J., and B. Gjerde. 2000. Isolation and enumeration of *Giardia cysts*, *Cryptosporidium* oocysts, and *Ascaris* eggs from fruits and vegetables. J. Food Prot. 63:775–778.

[42] Robertson, L. J., and B. K. Gjerde. 2001. Occurrence of parasites on fruits and vegetables in Norway. J. Food Prot. 64:1793–1798.

[43] Robertson, L. J., G. S. Johannessen, B. K. Gjerde, and S. Loncarevic. 2002. Microbiological analysis of seed sprouts in Norway. *Int. J. Food Microbiol*. 75:11–126.

[44] Robertson, L. J, Greig, J. D, Gjerde, B, Fazil, A. The potential for acquiring cryptosporidiosis or giardiosis from consumption of mung bean sprouts in Norway: a preliminary step-wise risk assessment. Int J Food Microbiol 2005; 98(3): 291-300.

[45] Said, D. E. S., Detection of parasites in commonly consumed raw vegetables. Alexandria Journal of Medicine. 2012; 48:345-352.

[46] Saki, J, Asadpoori, R, Khademvatan, S. h. Prevalence of intestinal parasites in vegetables consumed in Ahvaz, south west of Iran. *J Med Sci* 2013; **13**: 488-92.

[47] Sarkari, B. The study of parasitic contamination of vegetables in the city of Yasuj. *Armaghane Danesh J Yasuj Univ Med Sci* 1996; **4-3**: 31-7.

[48] Shafa-ul-Haq, A., Maqbool, A., Khan, U.J., Yasmin, G. and Sultana, R. (2014) Parasitic Contamination of Vegetables Eaten Raw in Lahore. Pak. *J. Zool.* 46:1303-1309.

[49] Tefera, T, Biruksew, A, Mekonnen, Z, Eshetu, T. Parasitic contamination of fruits and vegetables collected from selected local markets of Jimma town, southwest Ethiopi. Int Sch Res Notices. 2014:382715. doi:10.1155/2014/382715.

[50] Thurston-Enriquez, J. A., P. Watt, S. E. Dowd, R. Enriquez, J. L. Pepper, and C. P. Gerba. 2002. Detection of parasites and microsporidia in irrigation waters used for crop production. J. Food Prot. 65:378–382.

[51] U.S. Environmental Protection Agency (USEPA), 2005. *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA. EPA-815-R-05-002. Available from: https://www.epa.gov/sites/ production/files/2015-07/documents/epa-1623.pdf

[52] U.S. Environmental Protection Agency (USEPA), 2006. National Primary Drinking Water Regulations: Long Term 2 Enhanced Surface Water Treatment Rule. Federal Register 71(3):654-686.

[53] Wegayehu, T., Tsalla, T., Seifu, B., and Teklu, T. "Prevalence of intestinal parasitic infections among highland and lowland dwellers in Gamo area, South Ethiopia," *BMC PublicHealth*, vol.13, no. 1, article 151, 2013.

[54] Zohour, A, Molazadeh, P. Prevalence of pathogenic parasites in consumed vegetables in Jiruft. *J Birjand Univ Med Sci* 2001; **8**: 10-3.