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Determination of the level of parasitic infection (Cryptosporidium and Giardia) of the vegetables marketed in Ilam city

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Abstract

Background: Infected with intestinal parasites is one of the most important health and economical problems, which could have different effects, such as diarrheal diseases or death associated. The purpose of this study was to determine the level of prevalence of Cryptosporidium and Giardia parasites in the vegetable marketed in Ilam city.

Methods: This study was performed on 280 samples of fresh vegetables and lettuce in Ilam. The samples were taken at the level of 500 grams from the places where vegetables and lettuce are sold. Micro liters of each sample was placed on the slide using automatic micropipette, and Logel and Zyl-Nelson stainings were performed in order to identify Cryptosporidium and Giardia.

Results: From 200 samples, 54 samples were contaminated to Cryptosporidium oocyte and 13 samples to Giardia cysts. From 80 lettuce samples also 32 samples were contaminated to Cryptosporidium oocyte, and 6 samples contaminated to Giardia cysts. The results showed that the overall infection was 37%. Infection with Giardia cysts was 6.8% and infection with Cryptosporidium oocyte was 30.7%, and Cryptosporidium infection rates in vegetables and lettuce were different. This difference was statistically significant ($P<0.05$).

Conclusion: As a result of this research it is determined that the prevalence of Giardia and Cryptosporidium in Ilam vegetables is significantly higher, and the contamination of lettuce is far greater. Therefore, authorities should be more attentive to the field of education and the control of parasitic diseases.

Keywords: Cryptosporidium, Oocyte, Giardia lamblia, Vegetables

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Introduction

Parasitic diseases are among the diseases that are closely related to personal and public health. Our country is not free from various parasitic infections and requires efforts to identify more pathogens and little known or unknown factors. Vegetable consumption, especially those that are eaten raw have a major role in the transmission of parasites (1). Vegetables can transmit oocytes and protozoan-cysts and eggs and larvae of worms, that can cause diseases in humans (2). Cryptosporidiosis and giardiasis are protozoan infections that can cause digestive disorders and chronic diarrhea in recent years in healthy and unhealthy individuals who have impaired immune systems. This disease is more common in patients who have normal immune deficiencies from birth to middle age. In cases the disease leads to severe weight loss in children and patients with immune deficiency and jeopardizes their lives assecondary infectious agent. Several studies have been conducted indifferent parts of the world to investigate vegetable parasites. In the studies that have been conducted in Turkey, Saudi Arabia, Vietnam, Nigeria, and India the rate of parasite infection in consumption of vegetables are respectively 6%, 16%, 26%, 36% and 44%, and maximum contamination have been reported in vegetables, cabbage, chives and watercress (3-6). Iran is one of the areas in which the prevalence of parasitic infections is noteworthy

(7). In most parts of Iran also, eating raw vegetables with meals is a current nutritional habit, that provide a significant parentage of essential vitamins and nutrients of the body and always expose people to parasites (8,9). In Iran also, during the past years, studies have been conducted in various cities and the results indicate that there is always the risk of parasitic infections for the consumption of raw, unwashed, and not disinfected vegetables. In Ardabil contamination was higher in vegetables, spinach and basil so that the cysts of Entamoeba coli, Giardia, eggs Tinia, Dicrocoelium, and Ascaris have the highest reported rate (10). In Tehran the highest rate of infection was in leek and parsley, and Giardia cysts, eggs Trichostrongylus and Ascaris were reported as the most parasitic (11). Due to the lack of accurate epidemiological study in Ilam, there is no complete information about the release of parasitic diseases. Vegetables which are supplied as nonstandard by the vendors in the city, downtown, and along the street play a major role in the pathogenesis of people. This study examines parasitic vegetables. There is the possibility of vegetables being contaminated in the fields due to using the wastewater and well water in irrigated vegetables and access of animals to farms due to lack offences around the fields. In this study parasitic infected vegetables are considered in Ilam city. Thus, identification of cryptosporidium and giardia infected vegetables marketed in Ilam can assist health authorities with thee exercise of control and finally promotion of public health.

Methods

This study was performed in the winter of 2013 on 280 samples of edible vegetables and lettuce in Ilam. First vegetable supplies of shops in the city were identified and then 500 g of samples were randomly taken from each of them. First the leaves of each sample was completely separated using disposable and clean gloves. Each sample was kept in a separate container, which contains three liters of water with 6 ml dish washing liquid for 8 hours. During this period, it was stirred regularly every half hour to facilitate these parathion of oocysts from the plant. After that, the dish was left for 1 hour until the isolated oocysts were deposited. Then the vegetable was removed gently and the dishwashing water content was fixed in place for 16 hours, to ensure the complete precipitation of oocysts (12,13).

In the next step, about 2 liters of water in the container were slowly discharged using an electric pump. The precipitate and the remaining water were passed from sieve No. 100 (one hundred pores per inch, every pore diameter 150 microns) which is buffered on two layers, to remove coarse particles. After integration the suspensions were centrifuged twice for five minutes at 1000 rpm, and the contents of each tube were enriched by adding physiology serum to a final volume of two cubic centimeters and the number of samples were inserted, and kept in the fridge until testing. In the next step, the samples were placed on a lam and add 10 microliters of each sample using an automatic micropipette and we added a drop of logel solution and then put a lamella on it. Now the lam is ready to identify and recognize giardia cysts (14).

In the next step, 10 µl of each sample were placed on the slide using an automatic micropipette. Expansion obtained after drying for 3 minutes in methanol fixation. And it was placed in cold carbol fuchsin dye and then was had with running water. The bleaching stage was done using a solution of acid methanol for 10 to 15 seconds. After being washed with running water, slides were placed at 0.4% malachite color for 30 seconds, and after washing, dried and examined under a microscope with lens No. 100. If there is contamination (see oocyst and Giardia cysts), the mean number of oocysts and Giardia cysts in at least 50 microscopic fields were counted and their total numbers calculated based on the initial volume of the suspension.

Results

In this study, according to Table 1, 280 samples of vegetables and lettuce were tested in laboratory. From the total samples, 105 samples were polluted, and 175 samples were not contaminated. The results showed that infection in all samples was 33.6%. Infection with Giardia was 6.8%. Infection with Cryptosporidium was 30.7%, with Giardia on lettuce 7.5%, and in vegetables 5.6%. Infection with Cryptosporidium on lettuce was 40% and on vegetables was 27%. According to Table 2, the overall infection in the form of vegetables and lettuce was different, and these differences were meaningful in terms of statistics. (chi-square test: df=1, $P < 0.05$) (prevalence of infection is higher in lettuce). In terms of infection with Giardia, the difference between the two types of vegetables and lettuce was not significant (chi-square test: df=1, $P > 0.05$). Infection with Cryptosporidium on lettuce and vegetables was different and this difference was statistically significant, (chi-square test: df =1, $P < 0.05$) (lettuce is more infected).

Discussion

In this research the total 200 samples of vegetables were taken, each of which is included (parsley, basil, leeks,

Table 1. General information, Cryptosporidium and Giardia contamination levels in vegetables consumed in the city of Ilam

	No. of samples infected with Cryptosporidium	No. of samples infected with Giardia	No. of samples of non-infected	Total
Vegetable samples	54	13	133	200
Lettuce samples	32	6	42	80
Total	86	19	175	280

Table 2. The overall infection rate in terms of types of vegetables (for both species)

Descriptions	Infection		Total
	Positive	Negative	
Number	59	101	200
%, According to lettuce/vegetable	29.5%	27.5%	100%
Number	35	45	80
%, According to lettuce/vegetable	43.8%	56.2%	100%
Number	94	186	280
%, According to lettuce/vegetable	36.6%	66.4%	100%

thorp, oregano, mint, watercress). Fifty-four samples were infected with Cryptosporidium oocyst, and 13 samples were contaminated with Giardia cysts. From 80 samples of lettuce, 32 samples were infected with Cryptosporidium oocyst, and 6 samples were infected with Giardia cysts. Totally, 86 samples contained Cryptosporidium oocyst, and 19 samples were contaminated with Giardia cysts.

The total numbers of samples infected with Giardia were 19 samples. As a result, the number of samples infected with Giardia cysts was 6.8% of the whole samples (the overall prevalence of Giardia). Based on the results of research done by Malakoutian et al in 2009 in the city of Kerman, contamination of different vegetables with Giardia cysts was reported 0.74%. In this study, scallion had the highest rate of infection, and basil and radishes had the lowest rate (15). But in the research conducted in Norway from 1999 to 2001 it was observed that 6% of infections were with Giardia cysts (16). The survey conducted in Ramhormuz by Arab and Rahdar, showed that the rate of prevalence of Giardia was detected about 4.6% in vegetables (17). In a study conducted in Isfahan city, 480 samples were collected from different fields in this city. Seven types of Giardia were observed in the edible vegetables (11). Depending on the type of used fertilizer (organic or chemical), drinking water, and transportation of animals on the farm, the type and the percentage of infection is different in different regions.

Cryptosporidium oocyst infection percentage of the whole samples was 30.7%, and the total number of infected samples were 86. Of these 86 samples, 54 samples were vegetables and 32 samples were lettuce. Accordingly the number of lettuce samples taken is much lower than those of vegetables, percentage of Cryptosporidium oocyst infection of lettuce is more than those of vegetables. In the study on Cryptosporidium infection of lettuce marketed indifferent areas of Shiraz city, in two seasons of spring and summer, the number of 47 samples from 200 samples of lettuce (23.5%) with Cryptosporidium infection were found positive (18). Overgaauw et al, analyzing 475 samples of various vegetables such as lettuce and beans, reported the rate of Cryptosporidium infection 6% in Norway. There searchers reported that the infection rate of beans, is higher than the lettuce infection rate. Separating the parasites from farms, they concluded that contamination of vegetables and decontamination were linked to the use of contaminated water (19). In another study Paula et al, by studying 60 samples of a self-service foodstuff in Nitro in Brazil, they proved that 16 cases (26%) were infected with Cryptosporidium, which was proved to be more contaminated than the previous study (20). In the present study, Giardia infection in lettuce is 7.5% and in vegetables is 6.5%. Cryptosporidium infection in lettuce is 40% and in vegetables is 27%. Cryptosporidium has a wide host range and infects not only humans but also infects other animals such as poultry, sheep, goats, cattle, otter, carnivorous and fish (21). Inpatients with impaired immune systems, 3.6% of patients with diarrhea, were reported to be infected with Cryptosporidium (22). Wastes of birds, animals, or humans are infected with Cryptosporidium on farmlands, if used, the infection is transmitted through fertilizer, and infected agricultural products such as vegetables. In addition to soil contamination, there is the possibility of transmitted contamination of water. There are many reports of outbreaks caused by Cryptosporidium and Giardia with the source of water pollution, that has been originated from leaking sewage or direct infection with the feces of human or animals.

Conclusion

In this study the levels of Giardia infection were less than Cryptosporidium. It might have different reasons, but because there is no completely effective treatment for Cryptosporidium, a wide range of hosting and asymptomatic carriers, resistan of oocysts to disinfectant agents, and increasing incidence of the disease involved. Also, the over all infection and Cryptosporidium infection is higher in lettuce. Bivalves such as lettuce, due to the large surface of the leaves, and the special form where the leaves are attached to each end, causes Cryptosporidium oocyst maintain their level and are more contaminated than other vegetables. The differences in terms of infection to Giardia in two types of vegetables and lettuce are not significant. According to this research findings and the role of vegetables in diets and the risk of transmitting infections to human, it is necessary to take substantial action in planting, harvesting, storage, transport, distribution, and consumption of vegetables and raising public awareness. According to the research, consumed vegetable of Ilam in the winter was supplied from Khuzestan. This issue justified further research in this area. Educating the health tips when washing vegetables, with the aim of reducing the number of oocysts and not destroying them is an important step in prevention of Cryptosporidium infection in human. Due to the importance of vegetables in our diet and the possibility of transmission of parasitic infections to human by vegetables, it is necessary to consider a plan that prevents the movement of domestic and wild animals in the farm land, because in addition to damage to agricultural lands, they may increase the probability of transmission of zoonotic parasitic diseases. The accumulated fertilizer for farms, which is uninfected (accumulated long as compost) can play a role in reducing contamination. According to

the results of this research, based on the high overall infection and also high levels of cryptosporidial contamination of lettuce than vegetables, when washing, lettuce must have been separated from leaves and washed well with anionic detergent and then bed is infected.

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Ethical issues
We certify that all data collected during the study are presented in this manuscript and no data from the study has been or will be published separately.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
MA conceived and designed the study. MTY, MD, MM, FS and ZG performed the literature search and wrote the manuscript. All authors participated in the data acquisition, analysis, and interpretation. All authors critically reviewed, refined and approved the manuscript.

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