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Investigation of Parasitic Contaminations of Vegetables Sold in Markets in the City of Tabriz in 2014

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Abstract

Parasitic diseases are among the most common problems in developing countries. Various parasitic forms such as cysts, larvae and eggs of the parasite are transmitted orally through the consumption of vegetables. So, the aim of this study was to evaluate parasitic contamination of fresh vegetables consumed in the city of Tabriz. This cross-sectional study was conducted for 3 months in 2014 in Tabriz. In this study, 1620 samples from 10 kinds of vegetables (leeks, basil, mint, chives, radishes, parsley, lettuce, watercress, tarragon and coriander) were collected from 54 vegetable shops. After the washing process, centrifugation, sediment preparation and staining, each sample was examined for parasitic contamination. The parasitic infection was observed in 316 (19.5%) which 141 samples (8.7%) was related to metazoan and 175 samples (10.8%) was related to protozoan. the highest contamination was observed in leeks and the lowest in radish. Most parasitic contaminations were related to *Ascaris eggs* and *E. coli* cyst and the lowest parasitic contaminations were related to *Heterophyes eggs*. It can be concluded that vegetables sold in Tabriz are potential sources of human contamination with parasites. It is suggested that the use of fertilizers of human source in agriculture should be avoided.

Keywords: vegetables, parasites, parasite infection, Tabriz

1. Introduction

Parasitic contamination has worldwide prevalence and wide distribution (Sharafi, 2012). Their adverse effects on health and socio-economic situations are getting more visible and have led to various studies in some regions of Iran (Sharafi, 2012). Parasitic infections can affect on humans via different ways (water, soil, food and vegetables) and can lead to health risk and effect such as gastrointestinal disorders, malnutrition, anemia, allergies, and sometimes can be life-threatening (Razavyoon, 2003; Arbabi, 2005). Over 40 million people suffer from parasitic infections in all over of world and more than 10% of the world's populations are at risk of infection of parasitic diseases (Tappeh, 2006; Abadi, 2003). *Giardia lamblia* parasites, *Ascaris* and amoeba play the largest role in such parasitic contamination (Sarasiabi, 2002). The World Health Organization reported in 1975 that 700 million of the world's population was infected with *Ascaris* (Daryani, 2003). Although the health-effective aspects of consumption of raw vegetables are clear and they have most important role in human diet; however, the raw vegetables are assumed to contaminate by various viral and parasitic pathogens and are considered to be as an important agent in incidence of parasitic disease (Ezatpour, 2013). Iran is one of the areas

with high parasitic contamination level; thus, the identification of parasitic contamination sources and the transmission and prevention methods of their spread are health priorities. Edible vegetables are always at risk to contaminate by different types of parasites during planting, harvesting and marketing and have the potential for transmission of infection to humans (Ebrahimzadeh, 2013). Although the use of chemical fertilizers in farming and agricultural activities has been thrived in recent years however, there is still interesting to utilize the human and animal fertilizers in some farms (Khosravi, 2008). The occurrence and outbreak of parasitic diseases caused by eating raw vegetables has been reported in most developed and developing countries (Kozan, 2005; Saki, 2013). In last years, several studies was conducted in different cities of Iran such as Yazd and Tehran to evaluate the parasitic contamination level of vegetables and they illustrates considerable level of these contamination in vegetables in mentioned regions (Saki, 2013; Gharavi, 2000). The results of these studies showed that consumption of raw, unwashed and un-disinfected vegetables are always at risk of parasitic infections. Contamination of vegetables with parasites and other microorganisms can be through excretion, wastewater and irrigation water. The use of wastewater for irrigation of vegetables is a major cause of vegetable contamination (Fallah, 2012). Therefore, the identification of parasitic contamination transmitted through vegetables in each region may help public health authorities in the control and prevention of these infections in the region. This study was conducted to investigate the parasitic contamination through the sold vegetables in Tabriz which this could be helpful for authorities to find out the contamination level and to design comprehensive monitoring and educational programs according the needs.

2. Materials and Methods

This cross-sectional study was conducted in 2014 for 3 months during the summer in the city of Tabriz. In this study, a total of 1620 samples from ten types of vegetables (leeks, parsley, lettuce, coriander, radish, spring onion, tarragon, basil, mint and watercress) were collected from 54 vegetable shops of the city. Each of the samples were separately placed in a bucket with 5 L of water containing 10 g anionic detergent for 30 min to remove larvae, eggs and parasitic cysts attached to the vegetables. Vegetable samples removed from buckets and were rinsed under water pressure several times in order to introduce parasitic components of vegetables into the bucket of water. The water in the bucket was left for about 24 h to sediment the parasites and other particles. The supernatant was removed and the sediments were collected at the bottom of small containers. The sediments of each sample related to each vegetable were centrifuged for 2 min at 2000 rpm. The zinc sulfate was added to the tube according to Fust method and finally, the slides were prepared for both supernatant and sediment per tube by Gram method. The slides were observed under a microscope. The obtained data were analyzed using SPSS 18 software.

3. Results

The results of this study showed that 316 samples had parasitic contamination which it is equal to 19.5% of the total samples. The contamination was observed among all type of vegetables. The most parasitic samples were determined in leeks with 60 positive samples (37%) and the parsley and basil with 45 positive samples (27.07%) were in the second place. Also, the lowest level of contamination was seen in radish with 9 samples, which is equal to 5.55% of the whole positive samples. The parasitic prevalence based on the type of vegetables is presented in Table 1. Furthermore, the values of the each parasites contaminated the vegetable are represented in Table 2. It is clear that the greatest number of parasites was related to *Ascaris eggs* with 76 cases (24%) in fresh vegetables consumed in the city of Tabriz. Vegetable contamination with *Taenia eggs* was in 25 cases (7.9%), *H. nana eggs* in 14 cases (3.8%), *Trichocephal eggs* in 8 cases and *Trichostrongylus eggs* in 7 cases as placed after *Ascaris eggs*. Among the metazoans, *Heterophyes eggs* with 2 cases and *Dicrocoelium eggs* with 4 observed cases in the vegetables had the lowest level. Among the pathogenic protozoans, *Entamoeba hystolytica cyst* with 36 cases (11.4%), and *Giardia cyst* with 29 cases (8.85%) had the highest contaminations. However, in the whole pathogenic and non-pathogenic protozoans, the most contaminated vegetables were observed with *E.coli cyst* which had 106 cases (33.5%). Among the protozoan and metazoan parasites, the most observed cases belonged to *E.coli cyst* and *Ascaris eggs*. In all the ten kinds of vegetables were observed *Ascaris eggs* and *E.coli cyst.*

Table 1.The frequency of infections in all the vegetables (162 samples for each vegetable)

Infection case / Vegetable	With contamination		Without contamination	
	Number	Percent	Number	Percent
Leek	60	37	102	63
Chives	14	8/65	148	91/35

Basil	45	27/7	117	72/3
Parsley	45	27/7	117	72/3
Lettuce	18	11/1	144	88/9
Watercress	36	22/2	126	77/8
Mint	23	14/2	139	85/8
Tarragon	32	19/75	130	80/25
Coriander	34	21	128	79
Radish	9	5/55	153	94/45
Total	316	19.5	1304	80/5

Table 2. The frequency of parasites isolated from a variety of vegetables

Vegetable / Parasite		radish	Coriander	Tarragon	Mint	watercress	lettuce	Parsley	basil	chives	leek	Numbers	Percent
Ascaris eggs	Metazoan	1	10	5	4	6	3	14	12	3	18	76	24/05
Trichocephal eggs	Metazoan	-	-	2	-	-	1	-	2	-	3	8	2/5
Taenia eggs	Metazoan	-	2	2	1	5	2	3	5	1	4	25	7/9
H. nana eggs	Metazoan	1	-	3	2	1	-	-	2	1	4	14	3/8
Oxyure eggs	Metazoan	-	1	-	-	3	-	-	1	-	-	5	1/25
Heterophyes eggs	Metazoan		-	1	1	-	-	-	-	-	-	2	0/65
Dicrocoelium eggs	Metazoan	1	-	-	-	1	-	2	-	-	-	4	1/9
Trichostrongylus eggs	Metazoan		-	2			2	2	1	-	-	7	2/25
Entamoeba histolytica cyst	protozoan	2	5	5	4	5	4	5	4	-	2	36	11/4
Giardia cyst	protozoan	-	3	2	4	4	1	3	4	-	8	29	8/85
E.coli cyst	protozoan	3	13	10	6	10	5	15	14	9	22	106	33/5
Isospora oocysts	protozoan	1	-		1	1		1	-		-	4	1/25
	Total	9	34	32	23	36	18	45	45	14	60	316	100

4. Discussion

Since the vegetable has important role and especial place in food and human diets; thus, the parasitic contamination level of vegetables can be very important. Most parasites observed in vegetables are *E.coli* cysts that exist in 33.5% of samples and they were in all the vegetables used in the present study. In a study conducted in Saudi Arabia, 35.5% of the parasite level was reported which it verifies the current study (Al-Binali, 2006). Also, our study is consistent with studies conducted in Zabol on vegetables (Soleimnanpoor, 2013); however, it does not correlate with studies in Ardebil (10%) and Qazvin (7%) (Shahnazi, 2009; Daryani; 2008). The present work indicated that about 19.5% of the 1620 samples had parasites contamination. Other conducted studies has also showed that there is various levels of contamination in all vegetables consumed in the country had parasites, as parasites in fresh vegetables consumed in Ahvaz (Saki, 2013), Tehran (Gharavi, 2000), Zabol (Soleimnanpoor, 2013), Yazd (Dehgani Frirozabadi, 2004) and Esfahan (Izadi, 2006) have been reported (15.5%, 65%, 30.4%, 13.76% and 2%, respectively). It can be observed that there is higher parasitic contamination than Tabriz in mentioned studies except in Isfahan. The contamination percentage in Zahedan, Kerman and Qazvin is higher than 30 % which these values are greater than parasites of vegetables in Tabriz (Ebrahimzadeh, 2013; Shahnazi, 2009 and Malakotian 2009). Parasitic infections of vegetables have also been reported in several countries such as Turkey and Nigeria which to be 36 and 5.9%, respectively (Erdogrul, 2005; Damen, 2007).

In our study, it was observed that the most parasitic infections are related to leek which it corresponds to the studies conducted in Qazvin and Zahedan (Shahnazi 2009; Ebrahimzadeh, 2013); however, other studies conducted in Saudi Arabia and Brazil has obtained different results so that the highest contamination was related to chives in Saudi Arabia and lettuce and cress in Brazil, (Al-Binali, 2006; Mesquita, 1999). In addition, the highest infestation of vegetables was observed in chives in Kerman (Malakotian, 2009). The reasons for more contamination of are the harvesting of this product, prolonged contact with the soil and application of fertilizer of human or animal source. The lowest infection rate was observed in the city of Tabriz for radish which it is

agreed with the studies conducted in the city of Kerman and Qazvin (Shahnazi, 2009; Malakotian, 2009). Lower contamination of radish compared to other vegetables can be due to this fact that this vegetable is harvested only once and its root is removed and primary leaves are not used. Because of more establishments of the parasites among the leaves, the possibility of contamination of vegetables such as radish is less.

Among the metazoan, *Ascaris* eggs (4.7%) was the most contamination agent which it was higher than the observed contamination level in other studies conducted in Yazd and Isfahan, the level of contamination by *Ascaris* eggs in these cities has been reported to be 2.7, 1.94 respectively (Dehgani Firozabadi, 2004; Izadi, 2006). Vegetable contamination with *Ascaris* eggs in other countries have been reported as follows: Libya (68%) (Abougrain, 2010), Saudi Arabia (16%) (Al-Binali, 2006), Turkey (14%) (Ulukanligil, 2001) and Nigeria (47.7%) (Alade, 2013). However, these measures are for positive cases of samples. In this study, 24% of positive samples are related to *Ascaris* eggs. Among other nematodes that have been separated from vegetables in the different studies, it can be mentioned that *Hymenolepis nana* eggs in Zahedan was 5% (Ebrahimzadeh, 2013), while Phasiola and Hymenolepis in Jiroft was 21% (Zohour, 2001).

The differences in parasitic contamination rate in the country are possible due to the effective factors such as irrigation, social dependent and economic factors. One of the reasons for the abundance of parasitic agents in the studied vegetables is the use of wastewater to irrigate the crops and vegetables (Izadi, 2006). After *Ascaris eggs*, *Taenia eggs* is the next place among metazoan in terms of prevalence. In the studies on metazoan level prevalence in Kerman and Yazd, the contamination percent was %3.5 and 6.38%, respectively. It is more than that of the current study which is equal to 25 positive samples of 1620 (1.5%) (Firozabad, 2004; Malakotian, 2009). But the result for the metazoan in cities of Isfahan and Ahvaz was less than the results of the present study. In these studies, they have been reported as 0.7% and 0%, respectively (Izadi, 2006; Saki, 2013). This metazoan in vegetables was obtained to be 3.5% in Ankara and it was 2% in São Paulo, Brazil which is more than obtained value in the present study (Kozen, 2005; Mesquita, 1999). Since the type of *Taenia eggs* cannot be distinguished according to worm eggs, it is likely to be *eggs* of *Taenia echinococcus* (of hydatid cysts agent in humans) and there is need for it to be discussed in this case.

Trichostrongylus *eggs* is a zoonotic parasite which it was observed in 7 cases (2.25%) in present work. in cases of severe infection with this parasite, the sensitivity and effects in humans can be eradicated by disinfection of vegetables and treatment of infected humans and animals. The contamination with this parasite has been reported to be 15.1% in Esfahan, 1% in Ardebil, 2.9% in Kerman, 2% in Qazvin and 6.2% in Saudi Arabia (Izadi, 2006; Daryani, 2008; Malakotian, 2009; Shahnazi, 2009; Al-Binali, 2006).

The results of the study in Tabriz are consistent with the results of conducted study in Yazd in terms of presence of *Dicrocoelium* eggs while these results are higher in Kerman (Firozabad, 2004; Malakotian, 2009). Since the contamination cannot be transmitted to humans by worm eggs, there are no worries in terms of disease transmission to humans. However the mentioned contamination shows the contamination of vegetables through animal fertilizers or surface waters, it can be important in terms of health.

E. histolytica cyst and Giardia cyst recorded the highest amount of parasitic infections among pathogenic protozoan but *E.coli* cysts had the highest amount among non-pathogenic and pathogenic protozoan which it is in accordance to the results of studies conducted in Zabol, Ardebil, Qazvin and Zahedan; *E. coli* cysts were found to be more than the other protozoans in vegetables in mentioned studies (Soleimnanpoor, 2013; Daryani, 2008; Shahnazi, 2009; Ebrahimzadeh, 2013). Although, the virulence of this parasite does not matter, but considering the fact that the mentioned contamination occurs as a result of the contamination of vegetables by human waste, it is of importance to human health.

In this study, Giardia cysts were observed in 8.85% of the contaminated vegetables which it is less than the amount reported in Isfahan and Kerman (10.6 and 22.5%, respectively) (Izadi, 2006; Malakotian, 2009), and it is more than the amount reported in Zabol, Kerman and Ardebil (Soleimnanpoor, 2013; Daryani, 2008; Malakotian, 2009). Since the study on parasitic contamination has greatest importance; therefore, many research has been conducted in different countries such as Turkey (5.5%), Norway (2%) and Nigeria (6.5%), and their results show that the contamination of vegetables with Giardia in these countries is lower than the results of our study (Erdogrul, 2005; Damen, 2007; Robertson, 2000). Since the Giardia parasite is zoonotic and its contamination has been reported in different parts of Iran, thus the crossing of animals through the farms or vegetable farms will increase the potential of surface water contamination (Izadi, 2006; Malakotian, 2009).

Today, the parasitic contamination and diseases are decreasing in our society along with the rest of the world due to the relative improvement in the general health, economic, social and agricultural condition, fairly substantial reduction in the prevalence of parasitic diseases and modern methods of garbage collection, repair of sewage

systems and relative elevation in education of different classes' people. Studies conducted in Turkey have shown that washing and disinfection of vegetables eliminates all parasitic infections, 12 out of 203 vegetable samples taken, were contaminated with parasite spores, which is equal to 9.5%, while after washing and sterilization of samples, vegetables did not have any infection (Kozan, 2005). Therefore, it can be concluded that any kind of vegetables transported from farms to cities, and from there to homes traditionally, can cause parasitic contamination of hands, kitchenware and home environment. One of the strategies for eliminating parasite contamination in vegetables is the use of anionic detergent to reduce surface tension, followed by disinfection with hypochlorite (Khosravi, 2008).

5. Conclusion

According to the results, vegetables consumed in the city of Tabriz, are a potential risk for human exposure to parasitic contamination and all vegetable samples have at least *Ascaris eggs* and *E.coli* cyst. Since people often consume raw and fresh vegetables; thus, this risk will increase. Hence, the proper training on disinfection before the use of vegetables and introduction of effective antiparasitic material may be considered as good approach to reduce and eliminate the parasitic contamination and diseases.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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