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Research Article

Investigation Of Parasitological Contamination In Leafy Vegetables In Kırıkkale Of Turkey

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A B S T R A C T

Background/aim: The aim of this study was to determine the parasite contamination in leafy and native leafy vegetables sold in public bazaar and markets in Kırıkkale.

Material and Methodes: The study was carried out with leafy vegetables collected from markets (n= 346) and native leafy vegetables from bazaars (n=554) as seasonal in Kırıkkale province between February 2012 and January 2013. A total of 900 leafy vegetables were examined. Each sample of leafy vegetables collected and washed with three different washing solutions seperately. The samples were examined (x100-400) under a light microscope for helminth eggs, protozoan.

Result and Conclusion: Overall results showed that 2.44% of investigated samples were found to be contaminated with 13 protozoan oocysts and 9 helminth eggs. The following parasites, with their respective prevalence, were found; *Eimeria* spp. (0.77%), *Isospora* spp. (0.66%), *Fasciola* spp. (0.22%), *Dicrocoelium dendriticum* (0.22%), *Toxocara* spp. (0.11%) and *Strongyle* type eggs (0.44%). The highest (33.33%) rate of contamination was found in *ekşimen herb* (*Rumex acetosella*) and the lowest (1.28%) in parsley. However, no parasite contamination were detected in red cabbage, *yemlik* (*Tragopogon longirostris* Bisch. var. *longirostris*), *madımak* (*Polygonum cognatum* Meissn), salsify (*Sinapis hieraciifolia*), *efelek* (*Rumex pulcher* L.), wormseed goosefoot (*Chenopodium anthelminthicum*) and cress. Tris-buffer-saline (TBS) was found to be the most efective wash solution used to detect the parasites. Seasonal contamination was detected during April, May, June, November and December. These results highlight the potential risk for public health in terms of transmitting protozoan oocysts and helminth eggs present in leafy and native leafy vegetable sand. And the importance of washing these vegetables properly, before consumption.

Key words: Disinfecting, Kırıkkale, leafy vegetables, parasite contamination, Turkey.

Ö Z E T

Amaç: Bu çalışmanın amacı Kırıkkale İl'i halk pazarı ve marketlerde satılan taze, yapralı sebzelerde parazit kontaminasyonunu belirlemekti.

Materyal ve Metot: Bu çalışma Şubat 2012 ve Ocak 2013 tarihleri arasında Kırıkkale İl'inde marketlerden alınan yapraklı sebzelerin (n:346) ve pazardan alınan taze yapraklı sebzelerin (n:554) toplanması ile yapıldı. Toplam 900 yeşil yapraklı sebze incelendi. Her sebze örneği ayrı ayrı toplandı ve üç ayrı yıkama solüsyonu ile ayrı ayrı yıkandı. Örnekler helmint yumurtası ve protozoonlar yönünden ışık mikroskobunda incelendi.

Sonuç ve Tartışma: İncelemelerin sonunda % 2.44 oranında toplam 13 protozzon oocsyti ve 9 adet helmin yumurtası görüldü. Tespit edilen parazitler ve sırasıyla yüzde oranları *Eimeria* spp. %0.77, *Isospora* spp. %0.66, *Fasciola* spp. %0.22, *Dicrocoelium dendriticum* %0.22, *Toxocara* spp. %0.11 and *Strongyle* tip yumurtalar % 0.44 olarak belirlendi. En yüksek kontaminasyon % 33.33 ile ekşimen (*Rumex acetosella*) bitkisindeyken, en düşük kontaminasyon 1.28% oranı ile maydonozda bulundu. Ayrıca kırmızı lahana, *yemlik* (*Tragopogon longirostris* Bisch. var. *longirostris*), *madımak* (*Polygonum cognatum* Meissn), tekesakalı (*Sinapis hieraciifolia*), *efelek* (*Rumex pulcher* L.), kaza ayağı (*Chenopodium anthelminthicum*) ve tere bitkilerinde hiç parazit kontaminasyonuna rastlanmadı. Parazitlerin belirlenmesinde en etkili yıkama solüsyonu Tris-buffer-saline (TBS) olarak bulundu. Sezonal parazit kontaminasyonu ise Nisan, Mayıs, Haziran, Kasım ve Aralık olarak tespit edildi. Sonuç olarak taze ve taze yapraklı sebzelerin kumlarından gelen protozzon oocystleri ve helmint yumurtaları halk sağlığı için risk faktörü olduğu ve tüketimden önce bu sebzelerin düzgünce yıkanması gerektiğine dikkat çekildi.

Anahtar kelimler: Dezenfeksiyon, Kırıkkkale, parazit kontaminasyon, yapraklı sebze, Türkiye.

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Introduction

The raw (fresh) vegetables are considered as the most important ingredient of a healthy diet. Increasing demand for salad vegetables and global transport of foods from various parts of the world, is likely to increase the risk of carrying different stages of the parasites to humans and surface contamination. Foods can normally become a potential source for human infection. The sources of contamination are usually listed as faeces, soil and water contaminated with faeces. In developing countries, it is indicated that using water resources contaminated with human and animal faeces to irrigate vegetables has a potential resposibility for the spread of parasite eggs. Pathogen and/or nonpathogen gut parasites are mostly transmitted via contaminated food, water, soil, arthropods and rarely from mother to offspring (Daryani et al*.,* 2008; Abougrain et al*.,* 2009; Al-Megrin, 2010).

Various studies in different parts of the world showed that the vegetables can be responsible for the transmission of protozoans oocysts and cysts like; *Giardia*, *Entamoeba*, *Cryptosporidium*, *Cyclospora*, *Toxoplasma*, *Isospora*, *Iodomoeba* and *Blastocystis* (Gharavi et al*.,* 2002; Erdogrul and Sener, 2005; Al-Binali et al*.,* 2006; Al-Shawa and Mwafy, 2007; Daryani et al*.,* 2008; Abougrain et al*.,* 2009; Uga et al*.*, 2009; Al-Megrin, 2010) and helminths like; *Schistosoma*, *Dicrocoelium*, *Fasciola*, *Hymenolepis*, *Taenia*, *Toxocara*, *Ascaris*, *Enterobius*, *Trichostrongylus*, *Strongyloides* and hookworms (Gharavi et al*.,* 2002; Yakhchali and Ahmadiashtiana, 2004; Erdogrul and Sener, 2005; Al-Binali et al*.,* 2006; Al-Shawa and Mwafy, 2007; Daryani et al*.,* 2008; Abougrain et al*.,* 2009; Uga et al., 2009; Al-Megrin, 2010).

The increased demand of food, intensive internatinal transport of products and the presence of susceptible individuals are considered as the main resources of food-borne parasitic infections and raw or undercooked vegetables are responsible for the transmission to human (Gharavi et al*.,* 2002; Erdogrul and Sener, 2005; Kozan et al*.,* 2005; Tepe and Obek, 2006; Al-Shawa and Mwafy, 2007; Daryani et al., 2008; Abougrain et al., 2009; Al-Megrin, 2010; Uga et al., 2009). In addition, organic agriculture which has become quite common in our country. In recent years, organic agriculture causing more people to catch this type of pathogenic agents innocently. Fertilisation of the soil with faeces of infected animals emerges as a greater risk factor for human (Erdogrul and Sener, 2005; Abougrain et al., 2009; Al-Megrin, 2010). The aim of this study was to determine the parasite contamination in leafy and native vegetables sold in public bazaar and markets in Kırıkkale.

Materials and Methods

The study was carried out with leafy vegetables collected from markets (n= 346) and native leafy vegetables from bazaars (n=554) as seasonal in Kırıkkale province between February 2012 and January 2013. A total of 900 leafy vegetables were examined. Each sample of leafy vegetables collected was weighted as 100 g and chopped into small pieces and put in a sterile plastic bag and washed with three different washing solutions. 100 g chopped pieces were put in a sterile plastic bag and washed with each washing solutions seperately. The samples were examined (x100-400) under a light microscope for helminth eggs, protozoan oocysts and each parasite stage was identified according to Soulsby (1982). The protocol for each washing solution is described below.

1. Investigation with saline solution (0.95 % NaCl)

100 g chopped pieces were put in a sterile plastic bag and washed in 0.95% NaCl solution for overnight. After removing bits of leaves, supernatant was discarded and 5 ml of remainder washing water was transferred to test tubes and centrifuged at 2000 g for 20 min. After centrifugation, the supernatant was discarded and the sediment was put on three slides and a drop of lugol iodine solution was added and slides were examined under the light microscope (x100-400) for the presence of oocysts and eggs (Gharavi et al*.,* 2002; Erdogrul and Sener, 2005; Al-Shawa and Mwafy, 2007; Daryani et al*.,* 2008; Abougrain et al*.,* 2009; Al-Megrin, 2010).

2. Investigation with solution containing 1% sodium dodecyl sulphate and 0.1%Tween 80

100 g chopped pieces were put in a sterile plastic bag and later the samples were placed in a 1.5 lt detergent solution (containing 1% sodium dodecyl sulphate and 0.1 % Tween 80) and sonicated (Bandelin SONOREX RK 255 SH, Germany) for 10 min. Then 50 ml of the liquid obtained were transferred to centrifugation tubes and centrifuged for 15 min. at 1500 g (Kozan et al*.,* 2005). The sediment was examined (x100-400) under a light microscope.

Table 1. The prevalence of parasites in investigated vegetables.
Tablo 1. Araştırılan sebzelerde parazit prevalansı.

Vegetables	*Eimeri* pozitiv	*Eimeria* spp. pozitive %	*Isosporid* pozitive	*Isosporid* pozitive %	*Fasciola* pozitive	*Fasciola* pozitive %	*Dicrocoeliu* pozitive	*Dicrocoeliu* pozitive %	*Toxocar* pozitive	*Toxocara* pozitive %	*Strongil* type pozitive	*Strongil* pozitive %
Lettuce	4	4.04	0	0.00	1	1.01	0	0.00	0	0.00	0	0.00
Parsley	1	1.20	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Dill	1	1.21	1	1.21	0	0.00	0	0.00	0	0.00	0	0.00
Rocket	0	0.00	2	2.12	0	0.00	0	0.00	0	0.00	0	0.00
Red cabbage	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Leek	0	0.00	0	0.00	1	1.28	0	0.00	0	0.00	0	0.00
Green onion	0	0.00	1	1.05	0	0.00	2	2.10	0	0.00	2	2.10
Çıtlık	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	2.27
Yemlik (*Tragopogon logistris* Birch.	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Mustard (n=66)	1	1.51	1	1.51	0	0.00	0	0.00	0	0.00	0	0.00
Pursley	0	0.00	0	0.00	0	0.00	0	0.00	1	1.63	0	0.00
Water cress	0	0.00	1	2.94	0	0.00	0	0.00	0	0.00	0	0.00
Knottweed	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Salsify	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Efelek	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Oxalis	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Cress (n=3)	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Ekşimen otu	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	33.33
Toplam: 900	7	0.77	6	0.66	2	0.22	2	0.22	1	0.11	4	0.44

Table 2. The prevalence of parasites using solutions.
Tablo 2. Solusyonların kullanılması ile parazit prevalansı.

Solutions	*Eimeria* spp.		*Isosporid*		*Fasciola*		*Dicrocoeliu*		*Toxocara*		*Strongil*ty		Total	
	positi	positi	pos	pos	pos	positi	posi	posit	positi	posi	pos	pos	pos	pos
0.95 % NaCl water	2	28.57	1	16.	1	50	0	0.00	0	0.0	0	0.0	4	0.4
solution containing	3	42.85	3	50	0	0.00	0	0.00	0	0.0	2	50	8	0.8
Tris-buffer-saline	2	28.5	2	33.	1	50	2	100	1	100	2	50	10	1.1
TOTAL(n=900)	7	0.77	6	0.6	2	0.22	2	0.22	1	0.1	4	0.4	22	2.4

3. Investigation with washing solution Tris-buffer-saline (TBS)
The samples were washed with 150 ml of TBS (20 mM Tris base, 0.5 mM sodium chloride, %0.2 Tween 20 and double-distille water) in a 500 ml beaker. With the aid of a mechanical Shaker (Daihan SHO-2D), the beaker was shaken at 150 rpm for 30 min. The washing solution, was poured through filter into a sedimentation flask and left to sediment for 45 min. Then the supernatant was discarded and the sedimet put into a 15 ml centrifuge tubes. The tube was filled to 15 ml with TBS solution and centrifuged at 1500 rpm for 5 min. After the supernatant was discarded and a few drop of 10% formal-saline (10 ml formalin in 90 ml of 9% normal saline) to sedimet was added and the mixture was put on three slides and examined for parasites (Al-Binali et al., 2006).

Results

A total of 13 protozoans oocysts and 9 helminth eggs were detected in leafy vegetables. The parasites detected during the examination were, *Eimeria* spp. (0.77%), *Isospora* spp. (0.66%), *Fasciola* spp. (0.22%), *Dicrocoelium dendriticum* (0.22%), *Toxocara* spp. (0.11%) and *Strongyle* type eggs (0.44%) (Table 1). Contamination was detected in 2.44% of investigated samples (Table 2).

The highest (33.33%) rate of contamination was found in *ekşimen herb* (*Rumex acetosella*) and this is followed by green onion (5.26%) and lettuce (5.05%), mustard (3.03%), watercress (*Nasturtium officinale*) (2.94%), dill (2.43%), çıtlık (*Cichorium intybus* L.) (2.27%), rocket salad (2.12%) and purslane (1.63%). The lowest rate (1.28%) was in parsley.

However, no parasite oocyst and helminth eggs were detected in red cabbage, in *yemlik* (*Tragopogon longirostris* Bisch. var. *longirostris*), in *madımak* (*Polygonum cognatum* Meissn), in salsify (*Sinapis hieraciifolia*), in *labada* (*Rumex pulcher* L.), in wormseed goosefoot (*Chenopodium anthelminthicum*) and in cress (Table 1).

Contamination was observed in 0.44% (4/900), 1.11% (10/900) and 0.88 % (8/900) by %0.95-saline water, TBS wash solution (by Shaker) and 1.5 lt detergent solution (containing 1% sodium dodecyl sulphate and 0.1% Tween 80) (by Sonicator), respectively. The most affected wash solution from these solution was TBS (Table 2).

Seasonal contamination was detected in April, May, June, November and December months (Table 3).

Discussion

The rate of intestinal parasites were found to be higher in communities consuming high amounts of raw vegetables especially grown in farms fertilized with human waste and animal fertilizers (Ulukanligil et al., 2001; Erdogrul and Sener, 2005; Kozan et al., 2005; Al-Binali et al., 2006; Tepe and Obek, 2006; Kozan et al., 2007; Abougrain et al., 2009).

Besides many studies performed in other countries, only limited information exists concerning the importance of parasite contamination in leafy and native vegetables in Turkey (Ulukanligil et al., 2001; Erdogrul and Sener, 2005; Kozan et al., 2005). The parasite burden in vegetables were found to vary between 16% to 71% in countries like Saudi Arabia, Vietnam, Libya, Iran and Palestin-Gaza province (Gharavi et al.,2002; Al-Binali et al., 2006, Al-Shawa and Mwafy, 2007; Daryani et al., 2008; Abougrain et al., 2009; Uga et al., 2009; Al-Megrin, 2010). However, compared to these results, the contamination rate in unwashed vegetables decreased and ranged form none to 14% among different cities in Turkey (Erdogrul and Sener, 2005; Kozan et al., 2005). In this study, the contamination rate (2.44 %) in leafy and native vegetables was found to be lower compared to previous studies (Ulukanligil et al., 2001; Kozan et al., 2005). It is believed that this difference may be due to a developmental level.

As a result of several published studies, it can be concluded that parasite oocyts and eggs such as *Entamoeba coli*, *E. histolytica*, *Giardia* spp. *G. intestinalis*, *G. lamblia*, *Blastocystis hominis*, *Iodamoeba butschili*, *Toxoplasma gondii*, *Dicrocoelium* sp., *Taenia* sp., *Trichuris* sp., *T. trichura*, *Toxocara* sp., *T. canis*, *T. cati*, *Ascaridia galli*, *Fasciola* sp., *Hymenolepis* sp., *H. nana*, *Ancylostoma* sp., *Ascaris* sp., *Ascaris lumbricoides*, *A. duodenale*, *Ascaridia galli*, *Strongyloides stercoralis* and *Trichostrongylus* sp. can be found on green vegetables (Gharavi *et al*., 2002; Al-Binali et al., 2006; Al-Shawa and Mwafy, 2007; Daryani et al., 2008; Abougrain et al., 2009; Uga et al., 2009). In this study, 13 protozoans oocysts and 9 helminth eggs including *Eimeria* spp., *Isospora* spp., *Fasciola* spp., *Dicrocoelium dendriticum*, *Toxocara* spp. and *Strongyle* type eggs were detected in leafy vegetables.

Regarding the protozoan oocytys, *Eimeria* spp. (0.77%), *Isospora* spp. (0.66%) were observed in the present study. However, other protozoons like *Enterobius vermicularis*, *E. histolytica* and *Giardia* sp. were found in Turkey (Erdogrul and Sener, 2005; Kozan et al., 2005). This situation can be caused from animal fertilizer in the present study.

In previous studies, helmints such as *Taenia* sp. and *Toxocara* sp. were detected in Turkey (Erdogrul and Sener, 2005; Kozan et al., 2005). However, none of these parasites were detected in this study. Within the other helmint eggs detected in this study, the prevalence rate (0.22%) of *Fasciola* spp. and *Dicrocoelium dendriticum* as found to be idential. This prevalence rate was found to be lower than that of detected in previous studies, in which the rate of *Fasciola* spp. and *Dicrocoelium dendriticum* was found to be 14.5% and 28.9%, respectively (Al-Megrin 2010). It is considered that the cause of the different may be due to make widely free or intensive farming in the natural environment. The most important factor of the low rates observed for Turkey is intensive livestock.

Toxocara spp. is the most important zoonotic parasite and in this study, the contamination rate was found to be 0.11%. The rate of this parasite was 3% in Vietnam (Uga et al., 2009), 3.12% in Saudi Arabia (Al-Binali et al., 2006), between 18% - 26% in Tripoli-Libya (Abougrain et al., 2009) and 1.5% in Ankara, Turkey (Kozan et al., 2005). When compared to the previous

Table 3. Seasonal prevalence of parasites found in vegetables investigated between February 2012 and Ocak 2013.
Tablo 3. Şubat 2012 ve Ocak 2013 yılları arası araştırılan sebzelerde sezonal parazit prevalansı.

Investiagate period	Sample Numbers (% Infection)
February 2012	0/8(0.00)
April 2012	10/29 (34.48)
May 2012	3/52(5.76)
June 2012	2/31(6.45)
July 2012	0/24(0.00)
October 2012	0/21(0.00)
November 2012	3/53(5.66)
December 2012	2/55(3.63)
January 2013	0/39(0.00)

findings, *Toxocara* spp. was found to have a lower prevalence rate. As the reason for its care and feeding of cats and dogs and also shows that people are very conscious about the entry of stray animals in the area grown vegetables.

It is well known that food choice of communities among countries and traditions can be different from each other and this may result in consumption of several green leafy vegetable species. Previous studies showed that the observed contamination rate can vary among green leafy vegetable species like, leek and parsley (Gharavi et al., 2002; Kozan et al., 2005), dill (Daryani et al., 2008), lettuce (Al-Megrin, 2010) and green onions (Al-Binali et al., 2006), fenugreek (Yakhchali and Ahmadiasthtiani, 2004; Abougrain et al., 2009), rocket (Al-Shawa and Mwafy, 2007), Houttuynia sp. (Uga et al., 2009) and strawberries (Erdogrul and Sener, 2005). In this study, the highest rate (33.33%) of contamination has been found in *ekşimen herb* (*Rumex acetosella*), while the lowest rate (1.28%) was in parsley. The rate of contamination among green onion, lettuce, mustard, watercress (*Nasturtium officinale*), dill, çıtlık (*Cichorium intybus* L.), rocket salad and purslane ranged from 1.63% to 5.26%. However, no parasite contamination was detected in red cabbage, *yemlik* (*Tragopogon longirostris* Bisch. var. *longirostris*), *madimak* (*Polygonum cognatum* Meissn), salsify (*Sinapis hieraciifolia*), *labada* (*Rumex pulcher* L.), wormseed goosefoot (*Chenopodium anthelminthicum*) and cress.

The contamination rate of vegetables can vary among different methods using a variety of washing solutions. The washing solutions used to detect the parasite burden include high (95%) and low (0.85%) concentrations of NaCl (Gharavi *et al*., 2002; Erdoğrul and Sener, 2005; Daryani et al., 2008; Abougrain et al., 2009; Al-Megrin, 2010), sterilized or tap water (Yakhchali and Ahmadiasthtiani, 2004; Al-Shawa and Mwafy, 2007), tris-buffer saline with Tween (Al-Binali et al., 2006), detergent solution containing 0.5% Tween 20 and 1% sodium dodecyl sulfate (Uga et al*.,* 2009). In this study, three different washing solutions were investigated and TBS including %0.2 Tween 20 in the presence of mechanical shaking was found to be the most efective wash solution that can be used to detect the parasite contamination on vegetables. Other solutions like, 0.95% NaCl and detergent solution resulted in a lower prevalence of contamination like 0.44% and 0.88%, respectively. The validity of the method applied until the environmental contamination in the prevalence of parasites is very important, also.

The rate of contamination in vegetables were found to vary during the season. In countries like Saudi Arabia, the contamination was found to be higher during the dry season (December-April) and spring (Al-Binali et al., 2006; Al-Megrin 2010). The rate of parasite contamination also showed differences among different regions of Saudi Arabia, in which the contamination rate reached to the highest level during December and April in south-west part of the country (Al-Binali et al., 2006), while the contamination was higher (23.1%) in spring (rainy season) than that of in winter (9.9%) in Riyadh region (Al-Megrin 2010). In this study, the highest contamination was detected in April (34.48%) and the lowest in December (3.63%). In Hanoi region of Vietnam, in rainy season the contamination rate was found to be slightly lower (15%) compared to the dry season (17%) (Uga et al*.,* 2009). It has been suggested that the rain water might have washed the eggs on the surface of vegetables (Uga et al*.,* 2009). However, the obtained results in this study don't support the above hypothesis and from our observations it can be concluded that the rain has a positive effect on the parasite burden of vegetables detected in this study.

Conclusion

The results obtained in this study highlight the potential risk for public health in terms of transmitting protozoan oocysts and helminth eggs present in leafy and native leafy vegetable sand. And the importance of washing these vegetables properly, before consumption. However, related health care providers should be aware of this.

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