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RESEARCH ARTICLE

Detection of Helminth Egg Contamination on Raw Vegetables in Afyonkarahisar, Turkey

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ABSTRACT

This study aimed to investigate the helminth egg contamination of raw vegetables grown in Afyonkarahisar. The material of the study consisted of 508 randomly selected vegetable samples, including lettuce, parsley, carrot, green onion, spinach, cress, arugula, mint, dill, and purslane, which were collected from the field between October 2018 and September 2019. According to the relevant literature, the sediments obtained after the proper washing procedure were examined under a light microscope for helminth eggs. According to the results, *Taenia/Echinococcus* eggs were found in 2 (0.39%) vegetable samples, including 1 lettuce and 1 dill. *Toxocara* spp. eggs were found in 2 (0.39%) vegetable samples, including 1 lettuce and 1 mint. *Toxascaris leonina* eggs were found in 1 (0.2%) rocket sample, and hookworm/strongylid eggs were found in 58 samples (11.42%), including 4 lettuce, 6 parsley, 3 carrots, 18 green onions, 2 spinach, 9 cresses, 4 garden rocket, 6 mint, and 6 purslanes. Moreover, *Dicrocoelium* spp. eggs were detected in 2 (3,63%) carrots and 1 (2,04%) rocket, *Moniezia* spp. eggs were detected 2 (3.63%) carrots, *Fasciola* spp.eggs were detected in 1 (2.08%) green onion. This study concluded that some vegetables sold and consumed raw in Afyonkarahisar bazaars are contaminated with helminth eggs, which are a risk to public health. It was agreed that these vegetables should be thoroughly washed and consumed in accordance with hygiene standards, otherwise serious health problems may arise.

Keywords: Afyonkarahisar, Contamination, Helminth egg, Public health, Raw vegetables

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Afyonkarahisar'da Çiğ Sebzelerde Helmint Kontaminasyonunun Tespiti

ÖZ

Bu çalışma ile Afyonkarahisar'da yetiştirilen çiğ sebzelerin helmint yumurta kontaminasyonunun araştırılması amaçlanmıştır. Çalışmanın materyalini Ekim 2018-Eylül 2019 tarihleri arasında tarladan toplandığı haliyle semt pazarlarında satışa sunulan marul, maydanoz, havuç, yeşil soğan, ıspanak, tere, roka, nane, dereotu ve semizotu olmak üzere rastgele seçilmiş toplam 508 sebze örneği oluşturmuştur. İlgili literatürler doğrultusunda uygun yıkama prosedüründen sonra elde edilen sedimentler, ışık mikroskobunda helmint yumurtaları yönünden incelenmiştir. Buna göre, 1 marul, 1 dereotu olmak üzere 2 (%0.39) sebze örneğinde *Taenia/Echinococcus* yumurtası, 1 marul, 1 nane olmak üzere 2 (%0.39) sebze örneğinde *Toxocara* spp., 1 (%0.2) roka örneğinde *Toxascaris leonina* yumurtası, 4 marul, 6 maydanoz, 3 havuç, 18 soğan, 2 ıspanak, 9 tere, 4 roka, 6 nane, 6 semizotu olmak üzere 58 örnekte (%11.42) kancalı kurt/strongylid tip yumurta bulunmuştur. Ayrıca 2 (3.63%) havuç, 1 (2.04%) roka örneğinde *Dicrocoelium* spp., 2 (3.63%) havuç örneğinde *Moniezia* spp., 1 (2.08%) yeşil soğan örneğinde *Fasciola* spp. yumurtası tespit edilmiştir. Bu çalışmada Afyonkarahisar il pazarlarında çiğ olarak satılan ve tüketilen bazı sebzelerin halk sağlığı için risk oluşturan olan helmint yumurtaları ile kontamine olduğu sonucuna varılmıştır. Bu sebzelerin yeteri kadar yıkanması ve hijyen kurallarına uyularak tüketilmesi gerekmektedir; aksi takdirde ciddi sağlık sorunları ortaya çıkabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Afyonkarahisar, Çiğ Sebzeler, Halk Sağlığı, Helmint Yumurtası, Kontaminasyon

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INTRODUCTION

Fresh vegetables and fruits are vital components of a healthy and nutritious human diet. Vegetables and fruits have been reported to reduce the risk of developing diabetes, atherosclerosis, stroke and certain types of cancer if consumed regularly and are rich in vitamins, fibre, and minerals (Van Duyn and Pivonka 2000).

Heat-treatment of vegetables, like many other foods, gradually loses nutrients that are beneficial to human health, such as vitamins and minerals. Such loss is dependent on the amount and duration of heat exposure. Therefore, consuming vegetables raw for a healthy life has become more popular recently. Water and fertilizer sources, human and animal movement in areas where vegetables are grown are critical due to many pathogenic microorganisms that can contaminate vegetables and pose problems for public health.

According to the World Health Organization, parasites are ranked sixth among the most pathogenic factors causing infectious diseases in humans (WHO 2019). Vegetables consumed without following appropriate washing protocols are considered a critical source for the transmission of parasitic diseases to humans. It is reported that intestinal parasitic outbreaks in developed or developing countries may be related to the consump-tion of contaminated vegetables (Slifko et al. 2000).

Intestinal parasites can be transmitted via the faecal-oral route by consuming of contaminated food and water with the infective forms of the parasites. Faeces, irrigation water or sewage, are all possible sources of parasitic contamination (Al-Binali et al. 2006, Newell et al. 2010). Epidemiological studies show that vegetables are contaminated with parasite eggs, larvae and cyst forms due to irrigating vegetables with untreated wastewater in epidemic areas, and humans are infected after consuming raw contaminated vegetables (Kozan et al. 2005, Ahmed and Karanis 2018).

Ascaris lumbricoides, *Enterobius vermicularis*, *Trichuris* spp., *Toxocara* spp., Trichostrongylidae and hookworm infections were observed in humans after consumption of inade-quately washed vegetables and fruits as salads in catering establishments such as schools, hospitals, restaurants, and hotels (Vázquez et al. 1997, Mesquita et al. 1999, Coelho et al. 2001, Takayanagui et al. 2001).

This study was conducted in Afyonkarahisar, which is located on lands of Aegean region of Turkey. The primary source of income in Afyonkarahisar is agriculture and animal husbandry. Agriculture is mainly based on the cultivation of cereals like wheat, barley and sunflower. Poppy and sugar beet have an important place among the industrial crops. Legumes cultivation also plays an important role. Locally grown vegetables and fruits are offered for sale throughout the year in the district bazaars. No reports of parasite contamination of vegetables were detected following the literature search, including the "Afyonkarahisar" term. This study aimed to examine helminth contamination of vegetables grown in Afyonkarahisar, which are brought to the district bazaars and sold for raw consumption without any post-harvesting processing procedures.

MATERIALS and METHODS

Study Region

This study was conducted in Afyonkarahisar, which is located on lands of Aegean region of Turkey. Afyonkarahisar is located on the 38°45'25" north latitude and 30°53'87" east longitude (Figure 1). It has a hot and dry climate in the summer, while cold and snowy in winter. It is warm and rainy in the spring and autumn, and the precipitation is mainly in the form of rain. The average annual rainfall of Afyonkarahisar is 444 mm, and the annual temperature is 11 °C. The livelihood of Afyonkarahisar, in general, is based on agriculture and livestock. Therefore, domestic vegetables and fruits are sold in the district bazaars. Agriculture is primarily of smallholder type in the lowlands, which are located near the mountains. Alongside seasonal vegetable production, fruit production is also carried out to a large extent. Vegetable production is predominate in areas where irrigated farming is carried out. (Anonymus, 2019).

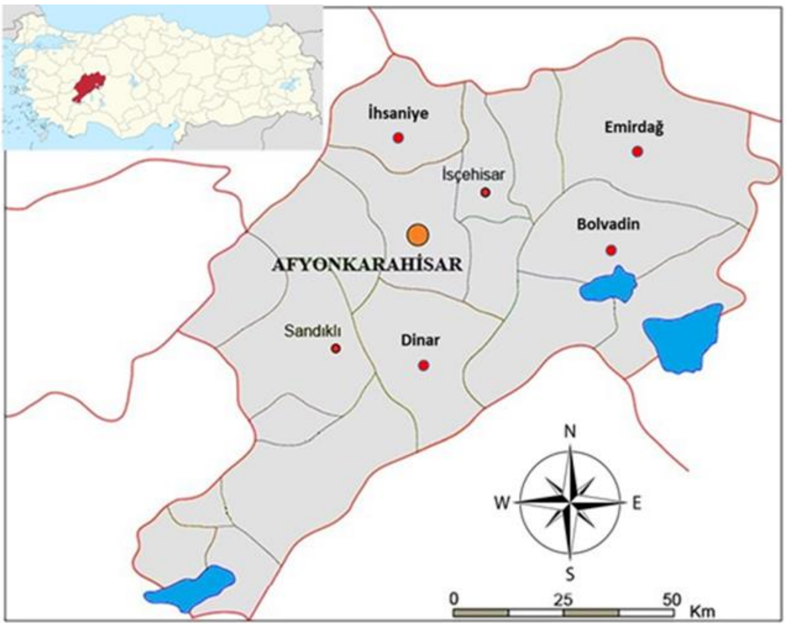

Figure 1: Study Region (Sarıışık 2021)

Sample Collection

A total of 508 samples consisting of lettuce, parsley, carrot, green onion, spinach, cress, arugula, dill, mint and purslane was collected from the established district bazaars every week between October 2018 and September 2019 on different days in Afyonkarahisar city centre and brought to the laboratory. These vegetables are mostly consumed raw as a salad.

Sample Preparation and Helminthological Examination

200 g of each vegetable was weighed in a plastic bag and washed with 2 l of physiological saline (0.95% NaCl) solution. The washing water was subjected to sedimentation for 24 h, and the upper layer of liquid was discharged, and the sediment centrifuged for 15 min at 2000 rpm. The supernatant was removed, and a drop of physiological saline containing lugol was added to the remaining sediment. The concentration of helminth eggs in the residue was investigated using the technique of Téléman Rivas modified by Bailenger (1962). Samples taken from the mixture were examined for contamination of helminth eggs using 10x and 40x magnification. Helminth eggs were detected according to laboratory manual (Bailenger 1962, Soulsby 1982, Ayres and Mara 1996, Hernández-Chavarría and Avendaño 2001).

Statistical Analysis

The chi-square test was used to compare the rates of egg types detected in the vegetable samples, and PASW Statistics 18.0.0 program was used in statistical analysis (SPSS 2009).

RESULTS

Helminth egg contamination in the examined vegetable samples is given in Table 1 and Figure 2. A total of 508 different samples made up of lettuce, parsley, carrot, green onion, spinach, cress, arugula, dill, mint and purslane were sold to the public for consumption in different retail markets in Afyonkarahisar and were analyzed for the presence of helminth eggs and larvae. The study results showed that 69 (13.58%) vegetables were contaminated with helminth eggs. These include 10% of lettuce, 9.23% of parsley, 12.71% of carrot, 39.58% of green onion, 4.4% of spinach, 16.07% of cress, 12.24% of rocket, 16.67% of mint, 2.32% of dill and 13.3% of purslane (Table 1). In green onion samples, Hookworm/Strongyle spp. eggs were significantly more than Fasciola spp. eggs ($P < 0.05$). In mint samples, Hookworm/Strongyle spp. eggs were significantly more than Toxocara spp. eggs ($P < 0.05$).

Out of the 60 samples of lettuce, 65 parsley, 55 carrots, 48 green onion, 45 spinach, 56 cresses, 49 rocket, 42 mint, 43 dills, and 45 purslanes examined, eggs of Hookworm/Strongyle spp. were detected in 6.67% of lettuce, 9.23% of parsley, 5.45% of carrot, 37.5% of green onion, 4.44% of spinach, 16.07% of cress, 8.16% of rocket, 14.29% of mint and 13.3% of purslane; Taeniid eggs in 1.66% and 2.32% of lettuce and dill, respectively; Toxocara spp. eggs in 1.66% and 2.38% of lettuce and mint, respectively; eggs of Toxascaris leonina in 2.04% of rocket only; Dicrocoelium spp. were detected in 3.63% and 2.04% of carrot and rocket respectively; Fasciola spp. were detected in 2.08% of green onion only and Moniezia spp. were detected in 3.63% of carrot only. Hookworm/Strongyle spp. eggs contaminated vegetables significantly more than the other helminth parasites($P<0.05$).

Table 1. Distribution of helminth egg detected in the examined vegetable samples in district bazaars of Afyonkarahisar.

Vegetables	Number of Samples	Hookworm/ Strongyle eggs (%)	Taeniid eggs	*Toxocara* spp. Eggs	*Toxascaris leonina* eggs	*Dicrocoelium* spp. eggs	*Fasciola* spp. eggs	*Moniezia* spp. eggs
Lettuce	60	4 (6.67[a])	1 (1.66 [a])	1 (1.66 [a])	0	0	0	0
Parsley	65	6 (9.23)	0	0	0	0	0	0
Carrot	55	3 (5.45 [a])	0	0	0	2 (3.63 [a])	0	2 (3.63 [a])
Green Onion	48	18 (37.5 [a])	0	0	0	0	1 (2.08 [b])	0
Spinach	45	2 (4.44)	0	0	0	0	0	0
Cress	56	9 (16.07)	0	0	0	0	0	0
Rocket	49	4 (8.16 [a])	0	0	1 (2.04 [a])	1 (2.04 [a])	0	0
Mint	42	6 (14.29 [a])	0	1 (2.38 [b])	0	0	0	0
Dill	43	0	1 (2.32)	0	0	0	0	0
Purslane	45	6 (13.3)	0	0	0	0	0	0
Total	508	58 (11.42 [a])	2 (0.39 [b])	2 (0.39 [b])	1 (0.2 [b])	3 (0.59 [b])	1 (0.2 [b])	2 (0.39 [b])

[a,b]Differences between odds with different letters on the same line are significant ($p<0.05$).

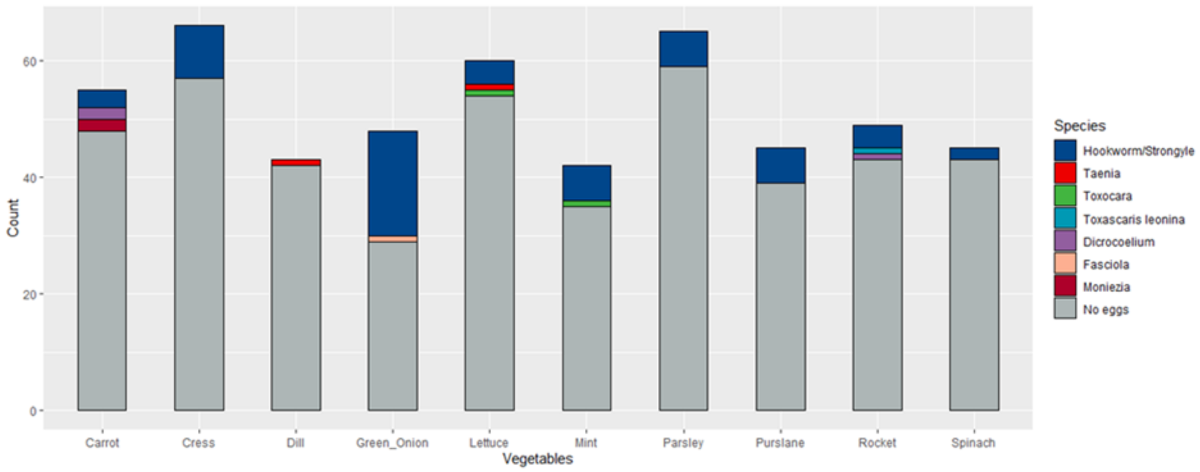

Figure 2: Distribution of helminth eggs in vegetable samples in district bazaars of Afyonkarahisar.

DISCUSSION

While 200-300 mm of the water required for vegetable production is generally met by direct precipitation, the rest is supplied from various water sources, including rivers, streams, lakes, wells and irrigation channels (Aras 2019). However, human or animal waste may mix with water sources from time to time. Animal fertilizers are also frequently used for vegetable cultivation. Both animal manure and water sources used for growing and irrigation of raw consumed vegetables could mediate the transmission of eggs belonging to various parasites of humans and animals. Some parasites like *Toxocara* spp. and *Taenia* spp. are zoonoses and pose significant risks to humans.

Five hundred eight vegetable samples were collected from the field and brought to the district bazaar without any cleaning It was identified in this study that 69 (13.19%) vegetable samples were contaminated with various helminth eggs. *Taenia / Echinococcus* spp., *Toxocara* spp. were found in companion animals, and helminth eggs which were seen in ruminant faeces like *Fasciola* spp., *Dicrocoelium* spp. and *Moniezia* spp. were detected in the studied samples. It is thought that infected ruminant faeces, which were not subjected to any decontamination processes, were used as fertilizer. In the case of insufficient water sources, the water required for plant vegetation can be supplied from wastewater or streams which might be contaminated with wastewater. Vegetables could be contaminated with various helminth eggs after irrigation with waste or untreated water, which is mixed with infected human or animal faeces.

Taeniid eggs were detected on one lettuce and one dill sample (0.39%) of the 508 vegetable samples, but no statistical significance was determined (P <0.05). Kozan et al. 2005 found Taeniid eggs in 3.45% of unwashed vegetable samples, and Adanır and Taşçı 2013 detected 2.7% of samples as contaminated. In other countries, Adamu et al. 2012 found six of 1130 vegetable samples (0.5%), Fallah et al. 2012, 28 of 304 vegetable samples (9.2%), Adenusi et al. 2015, 12 of 960 vegetable samples (1.25%) and Fallah et al. 2016 22 of 453 vegetable samples (4.86%) were contaminated with *Taenia/Echinococcus* eggs. Cysticercosis is caused by the larval stages of the parasite cestode *Taenia solium* infecting both humans and pigs. Ingestion of eggs which shed in the faeces of a human tapeworm carrier causes infection. These eggs are infectious and do not need a developmental stage outside the host. Humans are typically exposed to eggs by consuming of food or water contaminated with faeces containing these eggs or proglottids or through person-to-person transmission. *Taenia saginata* and *Taenia solium* are tapeworm located in the small intestine of humans. When *Taenia saginata* eggs are detected, it is thought that vegetables may have been irrigated with water contaminated with infected human faeces. However, it does not pose a risk to human health. Since there is no pork consumption in the region, *T. saginata* is ignored. While there is no evidence that human faeces contaminates the vegetable gardens, the presence of stray cats and dogs is expected in the study area. It is quite likely that the identified Taeniid type eggs belong to a tapeworm from the Taenidae family, which is found in the small intestine of cats and dogs: Echinococcus. The definitive hosts for *Echinococcus granulosus* are usually dogs, but many mammals and humans could be intermediate hosts, especially domestic ruminants (Eckert and Deplazes 2004). The infective eggs are released after disruption of segments in the faeces of dogs. Following consumption of contaminated water and food by intermediate hosts, the larval form, called a hydatid cyst, develops in various organs, especially in the liver and lungs. This has the potential to cause life-threatening disease. Therefore, the consuming of raw vegetables contaminated with Echinococcus eggs without proper washing increases the risk of developing hydatid cysts in humans (Daryani et al. 2008, Fallah et al. 2016). It is not microscopically possible to distinguish *Echinococcus* spp. based on egg morphology from other species in the Taenidae family. Therefore, it should not be ignored that these eggs seen in vegetable samples may also belong to important zoonotic *Echinococcus* spp. Ozkan et al. 2008 show that there were 2534 (13.13%) human cases in the Marmara region; 2114 (16.94%), in the Aegean region; 2578 (16.09%), Mediterranean region; 5404 (38.57%), in the Middle Anatolian region; 428 (5.70%), in the Black Sea region; 844 (6.80%), in the eastern Anatolian region; and 887 (2.75%), in the southeastern Anatolian region making a total of 14,789 cystic echinococcosis (CE) cases in Turkey. One hundred forty-two human CE cases were detected in Afyonkarahisar during 2001-2005 (Ozkan et al. 2008). In the study on the sero-epidemiology of the infection, Çetinkaya et al. detected CE 14.6% in 611 humans in Afyonkarahisar (Cetinkaya et al. 2005).

Ascarids are the most common gastrointestinal nematodes in cats and dogs. The embryo-developed eggs are excreted in the faeces of infected animals. They develop into an infective form that can survive for a few weeks to a few months in environments where temperature and humidity are favourable. Infective eggs are heat resistant from -25 °C to 36 °C and remain viable for at least one year (Overgaauw 1997). Oral intake of *Toxocara* spp. eggs is vital for human health due to the development of visceral or ocular larva migrans (Avcioglu et al. 2011). Therefore, Toxocariasis is considered one of the most important zoonoses in public health and economy (Macpherson 2013). *Toxocara* spp. eggs were found in 2 vegetable samples (0.39%), including one lettuce and one mint, and *Toxascaris leonina* egg was found in one rocket (0.2%), but no statistically significant difference was detected (P <0.05). In other studies, Kozan et al. 2005 reported that *Toxocara* spp. eggs were detected in 1.48% of their examined vegetable samples, while Avcıoğlu et al. 2011 detected in 1.0% of their samples, and Adanır et al. 2013 in 2.7% of the samples in Turkey. The percentage of vegetable samples reported being contaminated with *Toxocara* spp. eggs was 3% in Vietnam, 48.3% in Nigeria, 19.2% in Poland and 2.6% in Thailand, respectively (Uga et al 2009, Kłapeć and Borecka 2012, Maikai et al. 2013, Punsawad et al. 2019). Altındiş et al 2004. detected 5/4878 A. lumbricoides during 2000-2003 in Afyonkarahisar, Ak et al. 2006 detected *Ascaris lumbricoides* 4.8% in the Southeast Anatolian Region of Turkey during 2001-2003, Ulukanlıgıl et al. 2003 detected 64% in shantytown schools, 45.3% in apartment schools, 41.4% in rural schools in Şanlıurfa in 2000 (Ulukanligil et al. 2003, Altindiş et al. 2004, Ak et al. 2006). It is thought that differences between the studies could be due to the variation in the number of samples used in the study, and the geographical conditions and the growing conditions of these vegetables.

As for *Taenia* spp., it is not possible to distinguish microscopically *Toxocara canis* and *T. cati* eggs. Therefore, it can not be said that the eggs noticed in vegetable samples are either *T. canis* or *T. cati* eggs. The distinction of Toxocara eggs could be performed using molecular biological methods (Blaszkowska et al. 2011).

Strongyle type eggs with a thin shell, and oval shape, containing many blastomeres are produced by most nematodes in the Trichostrongylidae and Strongyloides family (Toparlak and Tüzer 2000). Except for some of the nematode species in these families, distinguishing the identity of the species by egg morphology alone is very difficult due to similarity in size, shape, character and appearance (Purwati et al. 2017). In this study, strongyle eggs were detected in 58 (11.42%) of 508 vegetable samples. These eggs could have included *Ancylostoma* spp, which is very important in public health. Considering the growing conditions of vegetables, it is thought that these eggs on contaminated vegetables may belong to various parasites in the Trichostrongyloidea and Strongyloidea families. It should not be ignored that vegetables could be contaminated with strongyle type eggs of zoonotic parasites due to using ruminant faeces as fertilizer without any process and defecation of stray cats or dogs in the areas where these vegetables are grown. Carnivore sheded hookworm eggs could develop to infective larvae within one week under optimum conditions and maintain their infectivity for a long time. After bare hand contact with vegetables contaminated with these larvae, cutaneous larva migrans can occur, caused by the larvae penetrating the skin and migrating under the skin surface (Toparlak and Tüzer 2000). Therefore, hands should be washed well after touching vegetables with bare hands, and hygiene rules should be followed.

CONCLUSSIONS

In conclussion, it was determined that some vegetables collected from the field in Afyonkarahisar and brought to the district bazaars without being subjected to any cleaning process, and consumed raw, were contaminated with different types of helminths. It is thought that carnivore animals enter the areas where these vegetables are grown, or that infected human or animal faeces reach these areas directly and via contaminated irrigation waters, causing contamination in vegetables. If vegetables are not properly washed, it will be inevitable to be exposed to significant zoonotic diseases in humans. For this reason, the use of fertilizers should be in accordance with guidelines, stray animals should be prevented from entering these areas, raw consumed vegetables should be washed properly before consumption and hygiene rules should be followed.

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