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Determination of Parasitic Contamination in Vegetables Collected from Local Markets in İzmir Province, Türkiye

Türkiye'nin İzmir İlindeki Yerel Marketlerden Toplanan Sebzelerdeki Parazit Bulaşının Belirlenmesi

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ABSTRACT

Objective: Fresh vegetables are an important part of a healthy and nutrient-rich diet but the consumption of raw vegetables without proper washing is the main way for transmission of parasites. This study was aimed at determining the rate of parasitic contamination in prewashed fresh vegetables sold at randomly selected 10 retail markets which is the last step to reach the consumer in İzmir, Türkiye.

Methods: A total of 80 samples selected from eight types of vegetables including tomato, spinach, lettuce, rocket, mint, parsley, dill, and cucumber were examined for parasitic agents microscopically by sedimentation method after washing samples with normal saline. Statistical analysis was performed using SPSS software version 20.0.

Results: Protozoan cysts, helminth eggs, and larvae were detected in 21 (26.2%) of 80 samples from eight different vegetable species. Rhabditidiform larvae 18.7%, *Blastocystis* spp. 5%, *Toxocara* spp. 2.5%; *Ascaris* spp., *Fasciola* spp., *Entamoeba histolytica/Entamoeba dispar* and hooked worms were found in 1.2%. Spinach and mint samples were contaminated with parasites significantly more than other fresh vegetable samples ($p<0.008$, odds ratio =80.0; $p<0.017$, odds ratio =46.6 respectively). *Cruznema* spp., a plant nematode, was found at the highest rate according to the results of culture, polymerase chain reaction and sequencing, respectively.

Conclusion: In this study, the parasitic contamination was found in approximately one of the four vegetables sold in randomly selected markets in İzmir. These findings show that vegetables sold in local markets can cause parasitic infections if they are consumed without adequate washing and awareness should be raised on this issue. In addition, it was concluded that morphological examinations should be confirmed by molecular studies and sequencing as much as possible in order to avoid misdiagnosis of rhabditidiform larvae.

Keywords: Vegetable, parasitic contamination, İzmir, Türkiye

ÖZ

Amaç: Taze sebzeler sağlıklı beslenmenin önemli bir parçasıdır, ancak parazitle kontamine çiğ sebzelerin uygun şekilde yıkanmadan tüketilmesi, paraziter enfeksiyonların bulaşmasında en yaygın yollardan biri olarak bildirilmiştir. Bu çalışmada Türkiye'nin İzmir ilinden rastgele seçilen 10 perakende markette satılan ön yıkama uygulanmış taze sebzelerdeki paraziter kontaminasyon oranının belirlenmesi amaçlandı.

Yöntemler: Domates, ıspanak, marul, roka, nane, maydanoz, dereotu ve salatalık olmak üzere sekiz çeşit sebzeden seçilen toplam 80 örnek, serum fizyolojik ile yıkandıktan sonra sedimantasyon yöntemi ile mikroskobik olarak paraziter etkenler açısından incelendi. İstatistiksel analiz, SPSS yazılımının 20,0 sürümü kullanılarak yapıldı.

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Türkiye PARAZİTOLOJİ Dergisi

Bulgular: Sekiz farklı sebze türünde toplam 80 örneğin 21'inde (%26,2) protozoa kistleri, helmint yumurta ve larvaları saptandı. Rhabditidiform larva %18,7, *Blastocystis* spp. %5, *Toxocara* spp. %2,5; *Ascaris* spp., *Fasciola* spp., *Entamoeba histolytica/Entamoeba dispar* ve çengelli solucan ise %1,2 oranlarında bulundu. Ispanak ve nane örnekleri diğer taze sebze örneklerine göre önemli ölçüde daha sık parazitlerle kontamine olduğu saptandı (sırasıyla p<0,008, olasılık oranı =80,0; p<0,017, olasılık oranı =46,6). Bu çalışmada sırasıyla kültür, polimeraz zincir reaksiyonu ve dizileme işlemleri sonucuna göre en yüksek oranda bir bitki nematodu olan *Cruznema* spp.'ye rastlandı.
Sonuç: Bu çalışmada, İzmir ilinde rastgele seçilen marketlerde satılan sebzelerden yaklaşık dört tanesinden birinde, paraziter kontaminasyon olduğu bulundu. Bu bulgular, yerel marketlerde satılan sebzelerin yeterince yıkanmadan tüketilmesi durumunda paraziter enfeksiyonlara neden olabileceğini ve bu konuda farkındalık oluşturulması gerektiğini göstermektedir. Ayrıca, rhabditid larvalarının yanlış tanısından kaçınmak için morfolojik incelemlerin mümkün olduğunca moleküler araştırmalar ve dizileme yöntemi ile doğrulanması gerektiği sonucuna varıldı.
Anahtar Kelimeler: Sebze, paraziter kontaminasyon, İzmir, Türkiye

INTRODUCTION

Vegetables contain vitamins, minerals, and phytochemicals that are antioxidants, phytoestrogens, and anti-inflammatory agents, which make them a crucial part of a healthy diet (1). Consumption of a variety of vegetables has significant health benefits such as weight control, reduction of the risk of obesity, stroke, cardiovascular diseases and protection against certain types of cancer (2).

In the last decade, healthier lifestyle choices have changed people's eating habits to incorporate more vegetables into their diets. This has created an increase in demand for fresh vegetables (3). However, if these vegetables are consumed raw without adequate washing, they may be important vehicles for the transmission of enteric bacterial, viral, and parasitic pathogens (3-5).

Various factors can be associated with the contamination of vegetables with pathogens. Insufficient treatment of wastewater or human and/or animal feces contaminated water supplies used for irrigation at farmlands can result in contamination of vegetables. Similarly, the use of untreated night soil as a fertilizer can also result in contamination (4). Ignoring hygiene rules during and after the harvest when handling (transport, storage, preparation, and processing) vegetables is also considered a source of contamination (4-6). Recently, epidemics of intestinal parasitic infections related to consumption of raw vegetables have been reported from both developed and developing countries (3,7,8). Geographic spread of these pathogens has been facilitated by growing global trade and increase in the number of susceptible individuals.

Fresh produce, if consumed with no or minimal preparation, has been implicated in several studies as a potential vehicle of transmission. Indeed, several protozoal cysts (*Entamoeba hystolytica/Entamoeba dispar, Giardia intestinalis, Entamoeba coli, Balantidium coli, Blastocystis* spp.), oocysts (*Cryptosporidium parvum, Cyclospora* spp., *Isospora belli*, *Toxoplasma* spp.) and helminth eggs and larvae (*Strongyloides* spp, *Trichuris trichiura, Enterobius vermicularis, Fasciola hepatica, Ascaris lumbricoides, Toxocara* spp., *Hymenolepis diminuta, Taenia* spp.) have been documented in Nigeria (4, 9), Sudan (5), Libya (7), Cameroon (8), India (10), Ethiopia (6,11), Egypt (12,13), Ghana (14), Malaysia (15), Iran (16), South Korea (17), Türkiye (18-22), Italy (23), Switzerland (24), Poland (25), Brazil (26), and Canada (27).

To our knowledge, there are several studies conducted on helminthic contamination of fresh vegetables and fruits in Türkiye (18-22). However, there is no study performed to determine the parasitic contamination of vegetables specifically in İzmir province. Therefore, the main objective of the present study was to evaluate the parasitic contamination of vegetables that were up for sale in İzmir's local markets for human consumption and the potential risks that these vegetables present to human health.

METHODS

Study Area

This study was carried out in İzmir city (38° 24′ 45″ N - 27° 8′ 18″ E), a province and metropolitan municipality of Türkiye in western Anatolia, situated along the Aegean coast. It is the third largest city in Türkiye in terms of population. Vegetables have an important place in the cuisine of the Aegean region of Türkiye.

Sample Collection

A total of 80 unwashed and pre-washed samples were purchased from randomly selected 10 wholesale markets and supermarkets in İzmir province. The commonly used samples consisted of tomato, spinach, lettuce, rocket, mint, parsley, dill, and cucumber. Only one sample was taken from each selected supermarket and the samples were chosen from readily found vegetables and then processed in raw condition.

Processing Samples for Parasitological Examination

The method used by Abougrain et al. (7) was slightly modified and used as follows: A sample of 100 g from each vegetable type was weighed and placed into a plastic bag. These samples were washed with 250 mL of physiological saline solution (0.85% NaCl) to detach the parasitic structures (ova, larvae and cysts) of protozoal and helminthic origin. Following an overnight sedimentation of the wash solution, the top layer was discarded and the remaining wash solution was centrifuged at 2000 rpm for 15 minutes. After centrifugation, supernatant was discarded. The sediment was examined in Lugol stained slides (six for each sample) under light microscope using x100 and x400 magnification. Identification of parasitic structures was based on their morphology (7).

Agar Plate Method and Molecular Diagnosis

Following microscopic examination, samples with nematodes were centrifuged and rhabditid larvae at the bottom were cultured by the modified Tahseen and Jairajpuri (28) method. Rhabditid larvae detected in sample sediments were cultured in autoclaved non-nutrient agar cultures of 1.5% concentration with human blood clot added as nutrient source for nematodes. The petri dishes were kept in a growth chamber at room temperature (28).

Larvae were obtained from agar cultures and stored in 70% ethanol at -20 °C prior to molecular processing. DNA extraction and rDNA amplification were carried out at the European Union Reference Laboratory for Parasites, Istituto Superiore di Sanità, Rome, Italy. Genomic DNA was purified from larvae using the

DNA IQTM Tissue and Hair Extraction Kit (PROMEGA, DC6740) according to manufacturer's instructions and the obtained DNA was amplified by multiplex polymerase chain reaction (PCR) (29,30). Briefly two sets of primer pairs were used in a multiplex PCR to amplify the region the 18S ribosomal RNA and the ITS1, 5.8S, ITS2 target loci (Table 1) (31,32). Reactions were performed in a final volume of 30 µL using Hot StarTaq Master Mix (Qiagen, Germany), primers (10 pmol of each primer), and 10 µL of DNA purified from a single larva. The amplification was performed as follows: an initial denaturation and polymerase activation step at 95 °C for 15 min, then 35 amplification cycles (denaturation at 95 °C for 10 sec, hybridization at 55 °C for 30 sec, and elongation at 72 °C for 30 sec), and a final elongation step at 72 °C for 3 min. The PCR was performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems, USA). PCR products were run on a 2% agarose gel in TAE (2M Tris-acetate, 50 mM EDTA, pH 8.3) buffer. The PCR products were sequenced by GATC Biotech (Germany) and obtained sequences were compared with the biological sequences in terms of regions of local similarity using GenBank with the BLAST system.

Statistical Analysis

Statistical analysis was performed using SPSS software version 20.0 for windows spreadsheet (SPSS Inc., Chicago, IL, USA). Pearson chi-square test was used to determine the association between the presence of parasitic contamination of tomato and the presence of parasitic contamination of other vegetables. A p-value <0.0001 was considered statistically significant difference.

RESULTS

A total of 80 fresh vegetable samples were examined for the presence of parasitic contamination. Helminth eggs and larvae, and protozoal cysts were detected in 26.2% (21/80) of fresh vegetables examined (Table 2). Vegetables that were found to be contaminated with at least one type of parasite included 80% (8/10) of the spinach samples, 30% (3/10) of lettuce samples, 70% (7/10) of mint samples, 10% (1/10) of parsley samples, and 20% (2/10) of dill samples. No parasitic structures were detected in the rest of the vegetable samples. Spinach and mint samples were contaminated with intestinal parasites significantly more often than other fresh vegetable samples ($p<0.008$, odds ratio =80.0; $p<0.017$, odds ratio =46.6 respectively).

The distribution of 25 parasites detected in 80 samples according to their species was detailed in Table 2; rhabditid larvae (18.7%) (Figure 1), *Blastocystis* spp. (5%), *Toxocara* spp. (2.5%) and *Ascaris* spp., *Fasciola* spp., *Entamoeba complex species (E. histolytica/ Entamoba dispar/Entamoba moshkovskii)*, hookworm with same incidence (1.2%).

Culturing of microscopically detected rhabditid larvae on standard blood agar plates resulted in the development of populations of rhabditid nematodes (Figure 2).

DNA amplification was successfully performed, targeting the ITS and *t 18S rRNA* gene. Edited sequences of ITS and 18S of these rhabditid nematodes were compared (BLAST) with the material

Table 1. PCR target genes and references used in the molecular investigations

Target gene	References	Primer pairs
18S ribosomal RNA	(31)	SSU18A AAAGATTAAGCCATGCATG SSU26R CATTCTTGGCAAATGCTTTCG
ITS1, 5.8S, ITS2 region	(32)	NC2 TTAGTTTCTTTTCCTCCGCT NC5 GTAGGTGAACCTGCGGAAGGATCAT

PCR: Polymerase chain reaction

Table 2. Overall distribution of parasitic contamination by species in fresh vegetables in İzmir

Vegetable (n=10)	Number positive (%)	No of detected parasites		Number of positivity							
		One	Two	Rhabditid larva	Hookworm egg	*Toxocara* spp. egg	*Ascaris* spp. egg	*Fasciola* spp. egg	*Entamoeba complex species**	*Blastocystis* spp. cyst	Total
Tomato	0	0	0	0	0	0	0	0	0	0	0
Spinach	8 (80)	6	2	8	0	0	0	1	0	1	10
Lettuce	3 (30)	2	1	0	0	2	1	0	0	1	4
Rocket	0	0	0	0	0	0	0	0	0	0	0
Mint	7 (70)	6	1	7	1	0	0	0	0	0	8
Parsley	1 (10)	1	0	0	0	0	0	0	1	0	1
Dill	2 (20)	2	0	0	0	0	0	0	0	2	2
Cucumber	0	0	0	0	0	0	0	0	0	0	0
Total (%)	21 (26.2)	17	4	15 (18.7%)	1 (1.2%)	2 (2.5%)	1 (1.2%)	1 (1.2%)	1 (1.2%)	4 (5%)	25 (30%)

**Entamoeba* complex *(E. histolytica/E. dispar/E. moshkovski)*

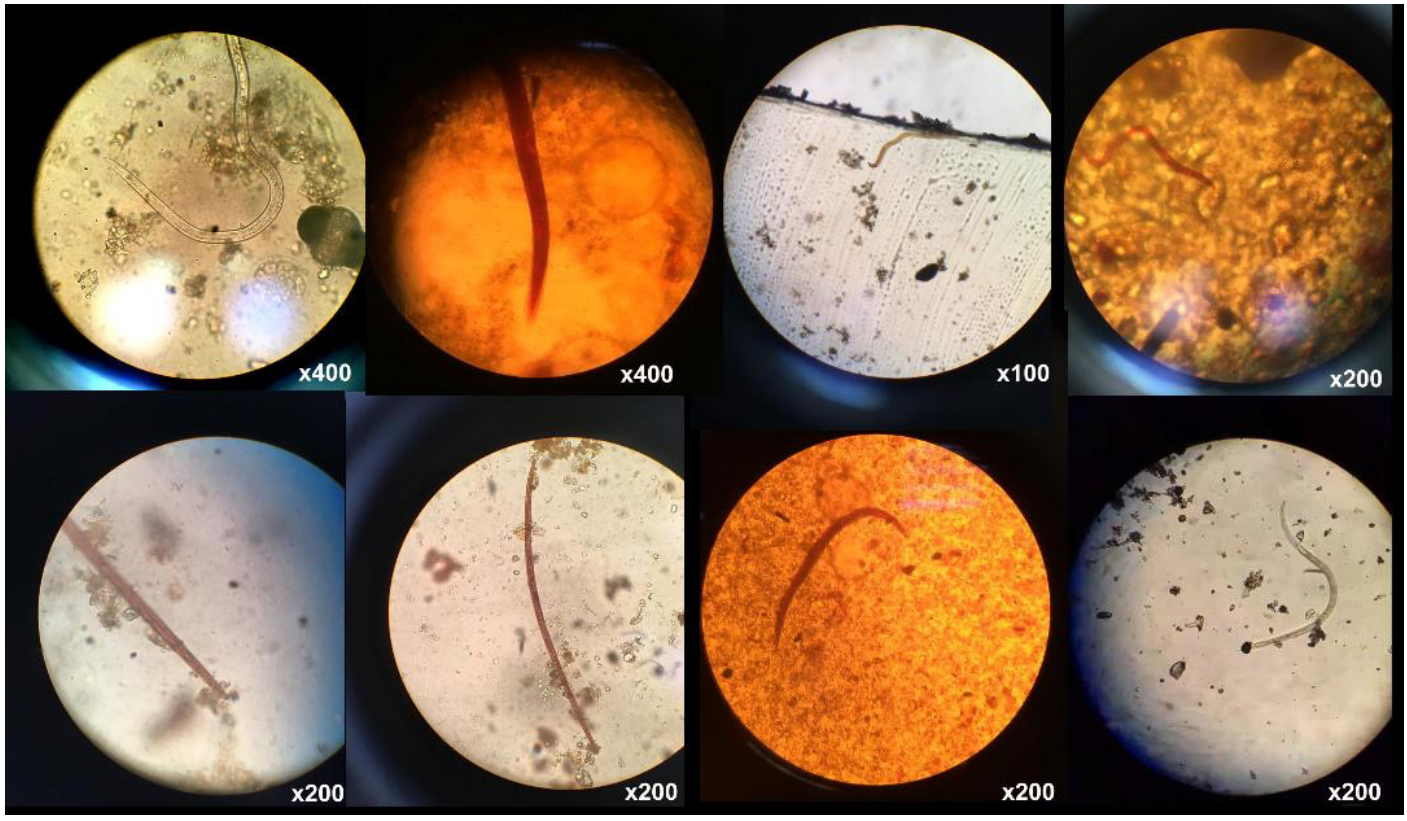

Figure 1. Microscopic appearance of rhabditiform larvae in preparations prepared with the native-Lugol method

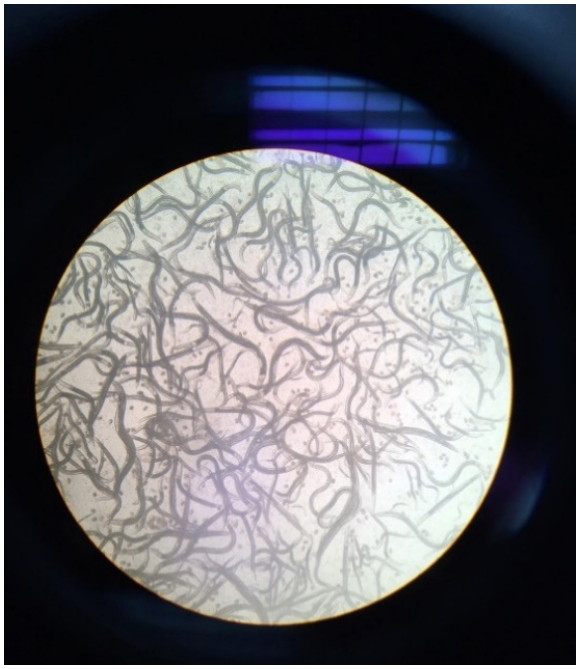

Figure 2. Microscopic appearance of rhabditiform larvae obtained by agar culture method

in GenBank and identified as *Cruznema* spp. (100% similarity on 750 bp sequence of 18S; 99% similarity on 145 bp of ITS1, GenBank accession numbers: AY284655.1 18S and MW228469.1 ITS1/5.8S/ITS2) (Figure 3).

DISCUSSION

The present study has shown that eight of the commonly consumed fresh vegetables in İzmir, Türkiye were contaminated with several parasitic structures. Similarly, studies worldwide have shown that raw vegetables could be vehicles for the transmission of intestinal parasitic infections (8).

Overall protozoal and helminthic contamination rate (26.2%) of fresh unwashed or pre-washed vegetables in our study was relatively higher than 13.5% in Khartuom, Sudan (5), 6.3% in Burdur, Türkiye (18), and 11.0% in Maiduguri, Northeastern Nigeria (33). However, it was lower than the rates found in the majority of studies such as 31.7% in Egypt (15), 59.7% in Iran (16), 54.4% in Ethiopia (11), 56.25% in Nigeria (9), and 58% in Libya (7). These variations in contamination rates in different studies can be attributed to geographical location, type and number of samples examined, laboratory methods used, water source and type used to irrigate produce, hygiene practices during harvest, handling methods after harvest, and the type of water used to wash vegetables (5). In addition, it was thought that the relatively low level of parasite contamination in our study may be due to the use of pre-wash, which can remove parasites from vegetables in the markets, and the fact that İzmir is a cosmopolitan city with a developed infrastructure system.

It has been postulated by some authors that the extent of contamination of vegetables and fruits is affected by their shape and surface texture. Parasite eggs, cysts and oocysts attach more readily to vegetables with rough surfaces compared to those with leathery surfaces due to their greater contact with the

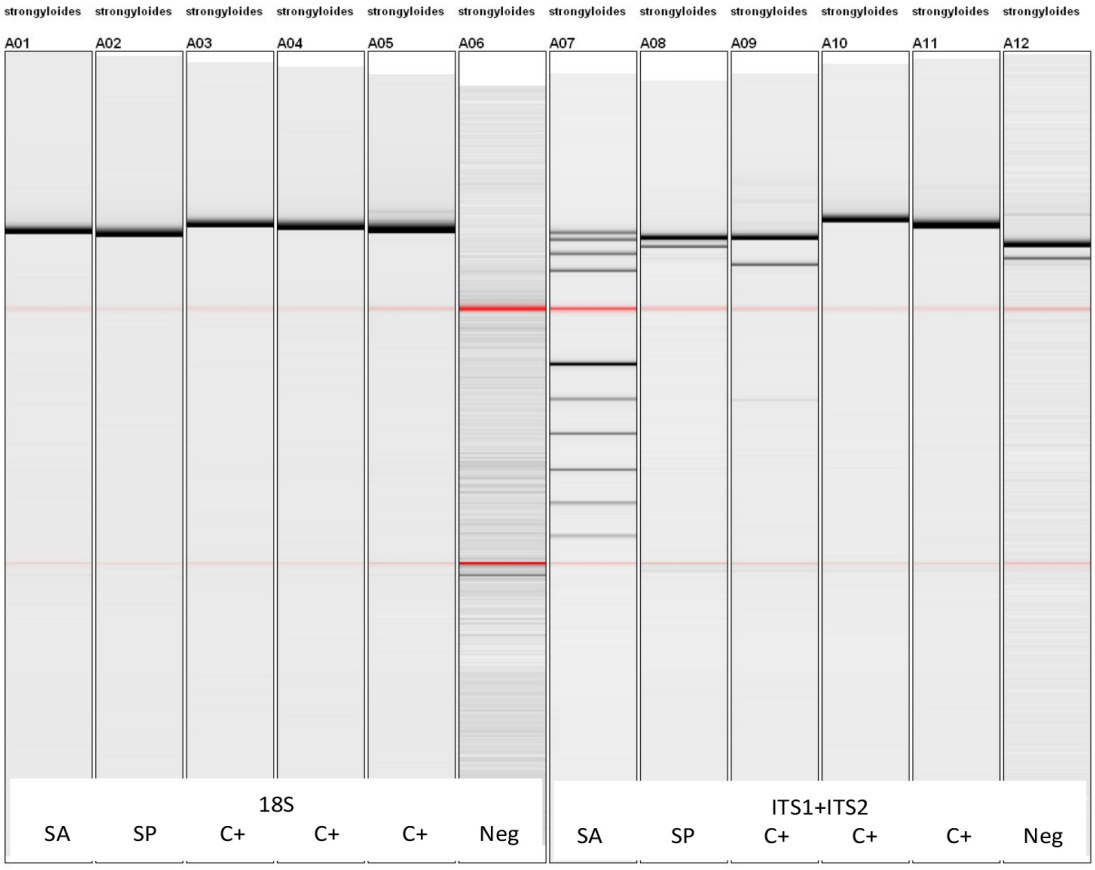

Figure 3. The agarose gel electrophoresis image of the PCR result [SA-Strongyloides from vegetables, SA 18S = *Cruznema* sp. (common bacterivorous soil nematode), 100% identity on 750 bp sequence, SA ITS = *Cruznema* sp. (common bacterivorous soil nematode), 99% similarity on 145 bp of ITS1]

PCR: Polymerase chain reaction, SP: Strongyloides from human stool

contaminated soil surface (4-6,13,34). This may explain why in the present study spinach, mint, and lettuce were contaminated the most (80%, 70%, and 30%, respectively), and tomato and cucumber had no contamination. It is also consistent with the results of other studies that showed the absence of parasites in leafy vegetables such as tomatoes, cucumbers and peppers (5,35,36).

In this study, the most frequently recovered parasite in vegetable samples was Rhabditid larvae (18.7%) which was initially thought to be *Strongyloides* spp. during superficial morphological examination. *Strongyloides* spp. infections are often at the forefront of diagnostic staff's minds because of their zoonotic potential. It is recommended that diagnosticians not to draw conclusions too rapidly, especially in unusual or unexpected infections (37). Since *Strongyloides* spp. infections are not endemic in Türkiye, we planned to perform further confirmatory tests before reporting rhabditid larvae as *Strongyloides* spp. After microscopic examination, agar plate culturing modified for the growth of pathogenic nematodes, PCR and sequencing were performed successfully and larvae was identified as *Cruznema* sp., a plant nematode.

Similarly, Stachurska-Hagen (37) described two apparent cases of rhabditid infections in dog and piglet. After examination with culturing, PCR and sequencing, *Pelodera* sp. and *Rhabditis* spp., were identified in dog and piglet, respectively. Nevertheless, environmental contamination with free-living nematodes following fecal shedding cannot be completely excluded (37). When molecular techniques are used for diagnosis or confirmation of morphological findings, it should be noted that sequencing of PCR products is the only way to ensure that other unexpected amplifications do not occur. Many publications (35,38-40) reported to have found *Strongloides stercoralis* as a result of their morphological examination of the larvae but a non-pathogenic species cannot be definitively ruled out without sequencing.

Similar to our study, *Blastocystis* spp. was found in the studies performed by Yusof et al. (15) and Caradonna et al. (23). It is expected to find *B. hominis* since humans are affected by these pathogens worldwide. *Toxocara* spp. was the third most frequently detected parasite in this study with a contamination rate of 2.5%. This rate is lower than those found in Ethiopia (6), Libya (7), and Nigeria (4). In Türkiye, the contamination rate of vegetables with *Toxocara* spp. was 2.7% in Burdur (18), 1.48% in Ankara (19) and 1% in Bursa (20).

No taeniid egg was detected in the samples examined in the present study. However, *Taenia/Echinococcus* species have been reported to contaminate vegetables in other studies. Contamination rate of taeniid eggs in fresh vegetables was 1.25% in Nigeria (4), 2.7% in Burdur, Türkiye (20), 25% in Libya (7), and 3.75% in Ankara, Türkiye (19).

Federer et al. (24) reported that microscopic identification could not be done for all samples because the samples contained large amount of dirt and pollen particles same size as taeniid

eggs. However, when multiplex PCR was performed on these particulate-containing samples, *Taenia* spp. and *Echinococcus* spp. DNA was detected. A notable finding is that *T. saginata* was identified in one of the samples of this study, which indicates contamination with human feces. Although *T. saginata* eggs are not infective to humans, this finding indicates that the vegetables are grown in unhealthy hygiene conditions. Such conditions create an increased risk for transmission of geohelminths such as *Ascaris* spp. or *Trichuris* spp., protozoa such as *Giardia* spp. and other bacterial or viral pathogens (41). *Ascaris lumbricoides* is also often used as a parasitological indicator to determine the quality of hygiene (10).

In studies using predominantly morphological identification, as in our study, it was aimed to detect parasitic contamination rather than pathogenicity of the parasite. Large-scale studies using advanced techniques including staining, cultivating, molecular technique such as squencing in addition to morphological detections are recommended for comprehensive identification of pathogenic parasitic contamination through subspecies identification. In addition, the risk factors associated with vegetable contamination are also needed to investigate in the futher studies.

CONCLUSION

The current study has shown that 26.2% of the examined vegetables had some sort of parasitic contamination. This highlights that some of the vegetables purchased from the local markets may pose significant health risks. In order to reduce transmission of parasites from vegetables to humans, strict hygiene practices must be followed, and all vegetables must be thoroughly washed before consumption. Furthermore, wastewater must be adequately treated, and preventative measures should be implemented to prevent contamination of water supplies with animal and human feces. Farmers and personnel who are involved in harvesting and post-harvesting handling must be educated in proper collection, storage, and transport of the vegetables and fruits to minimize the risk of contamination. It must be remembered that prevention of contamination of vegetables is the most effective way to reduce food-borne parasitic infections. In addition, advanced molecular techniques such as sequencing after morphological identification, should be performed in order to avoid misdiagnosis which is important to public health data.

* Ethics

Ethics Committee Approval: Ethics committee was not taken because no human or animal samples or data were used in our study.

Informed Consent: N/A.

Peer-review: Internally and externally peer-reviewed.

* Authorship Contributions

Concept: F.B., E.A.Ö., S.Ö.B., A.B., D.D.E., M.K., Design: F.B., E.A.Ö., S.Ö.B., A.B., D.D.E., M.K., Data Collection or Processing: F.B., E.A.Ö., S.Ö.B., A.B., D.D.E., M.K., Analysis or Interpretation: F.B., E.A.Ö., S.Ö.B., A.B., D.D.E., M.K., Literature Search: F.B., E.A.Ö., S.Ö.B., A.B., D.D.E., M.K., Writing: S.Ö.B.

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