Journal of Food Quality and Hazards Control 10 (2023) 13-20

Parasitic Contamination in Five Leafy Vegetables Collected from Open Marketplaces in Giza, Egypt

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HIGHLIGHTS

- Leafy vegetables had a very high parasite contamination rate (86%) in Giza, Egypt.
- Rate of contamination by protozoan parasites was higher than rate of contamination by helminth parasites.
- *Entamoeba histolytica/dispar* was the most encountered parasite with an estimated prevalence reaching 40.6%.

Article type
Original article

Keywords
Vegetables
Parasites
Helminths
Parasitology
Public Health
Egypt

Article history
Received: 6 May 2022
Revised: 7 Oct 2022
Accepted: 13 Nov 2022

ABSTRACT

Background: Leafy vegetables could be contaminated with parasitic infective stages, making them possible sources of human parasitic infections. This study aimed to assess parasites contamination level among five different leafy vegetables collected from open marketplaces in Giza, Egypt.

Methods: Hundred vegetable samples were collected from dill, parsley, coriander, rocca, and mint (20 of each). Samples were processed using sedimentation and concentration methods and the sediments were examined as wet smears either unstained or stained with Lugol's iodine. Also, modified trichrome and Ziehl-Neelsen stains were used to detect *Microsporidia* spores and coccidian oocysts. Statistical software SPSS version 20 was used for data analysis.

Results: A total of 86% of the examined samples were found contaminated with parasite's eggs, cysts, oocysts, and larvae. Coriander was the most contaminated vegetable with contamination rate of 95% while dill was the least contaminated (80%). Also, protozoan parasites contamination rate (77.1%) was higher than helminths parasites contamination rate (22.9%). *Entamoeba histolytica/dispar* was the most prevalent parasite (40.6%) followed by *Blastocystis hominis* (39.5%), *Ascaris lumbricoides* (18.6%), *Cyclospora* spp. (15.11%), *Giardia lamblia* (11.6%), *E. coli* (9.3%); while *Cryptosporidium* spp., *Iodamoeba bütschlii*, *Trichuris trichiura*, *Enterobius vermicularis*, *Fasciola*, and hookworms' eggs were the least prevalent parasites (1.1%).

Conclusion: Leafy vegetables in Giza, Egypt had an extremely high parasite contamination rate. So, consuming such vegetables without proper washing facilitates transmission of parasitic infections which could pose a major health risk to Egyptians.

Introduction

Many parasites may be transmitted through food. The most prevalent food-borne parasites in underdeveloped countries are intestinal parasites. Intestinal parasite infections pose a serious threat to public health all over

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To cite: El-Sayed N.M., Gawdat S.S., El-Kholy H.S., Elmosalamy A. (2023). Parasitic contamination in five leafy vegetables collected from open marketplaces in Giza, Egypt. *Journal of Food Quality and Hazards Control*. 10: 13-20.

DOI: 10.18502/jfqhc.10.1.11984 Journal website: http://jfqhc.ssu.ac.ir

the world, particularly in children where they result in diarrhea, malnutrition, anemia as well as some developmental disorders (El-Sayed and Ramadan, 2017a).

Leafy vegetables can help to improve nutritional status and avoid a variety of ailments. They are a rich source of vitamins, minerals, and dietary fibers, making them an essential component of a balanced human diet (Traoré et al., 2021). However, various human populations' eating habits and hygiene standards influence the chances of acquiring a parasite infection through food. Eating unwashed raw vegetables has been associated with the acquisition of several intestinal parasites, such as protozoa cysts and oocysts or helminths' eggs and larvae, which in turn increase the risk of food-borne illnesses (El-Sayed et al., 2021).

Parasitic contamination of vegetables has been related to several factors such as the use of untreated wastewater for irrigation, soil contamination with humans and animals' stools, and the use of improperly managed animals manure as fertilizer (Bekele et al., 2017). A moist environment is necessary for the growth of vegetables, and this moisture also aids in the development of several parasite species (Abougrain et al., 2010). Vegetables may become contaminated while being harvested, transported, stored, marketed, and otherwise processed in an unhygienic manner (El-Sayed et al., 2021).

Vegetables contamination by different parasites has been documented in several studies from different countries. The prevalence of contamination varies widely among different regions ranging from 15.1% in the United Arab Emirates (El Bakri et al., 2020) to 100% in Yemen (Al-Sanabani et al., 2016). The variations may depend on geographical locations, conditions of climate and environments, the type of vegetables examined, the used sample size and the used techniques for identification of the parasites in the samples (Tefera et al., 2014).

There have been relatively few studies focused on evaluating the parasite contamination of vegetables in various Egyptian regions (Ahmed et al., 2020; Etewa et al., 2017; Hassan et al., 2012). Determining the level of vegetables contamination may aid in the development of effective anti-parasitic control strategies to promote health and offer protection from diseases. In this regard, the present investigation was planned to assess parasites contamination level among five different leafy vegetables collected from open marketplaces in Giza, Egypt.

Materials and methods

Study area

This cross-sectional study was carried out between July and October 2020 using randomly selected vegetable samples from open marketplaces in Giza city, Egypt. Giza, a city of the Greater Cairo metropolis, is situated on the West bank of the Nile, 4.9 km Southwest of Central Cairo. It has a total area of 1,580 km². It has a population of 2.4 million. It has many areas which are under small and large-scale vegetable cultivation. The average maximum temperature in this agricultural region during the summer is 35.5 °C, while the average low is around 23.3 °C with an average humidity of 48%.

Collection of vegetable samples

In this study, 100 samples from five different green leafy vegetables-20 samples of each-were examined. The studied vegetables were dill (*Anethum graveolens* L.), parsley (*Petroselinum crispum*), coriander (*Coriandrum sativum*), rocca (*Eruca sativa*), and mint (*Mentha piperita* L.). These vegetables were chosen because they are regularly consumed raw and are widely available and sold in every market of the study area. Vegetables were purchased from randomly selecting street sellers and vegetable retailers. Following that, each sample was placed in a distinct plastic bag and identified by a unique number. Within 24 h of sample collection, each sample was processed and examined for parasites detection.

Samples processing

To separate the parasites from the vegetables, 100 g of each vegetable were soaked in 1,000 ml of physiological saline solution (0.9% sodium chloride) with agitation for 15 min. The washing solution was then let to stand on the bench overnight for optimal sedimentation. The supernatant was decanted on the next day, and the residual washing water was filtered through a strainer with a pore size of 300 µm to eliminate unwanted debris before being allowed to sediment for another hour. Then, the supernatant was discarded once more. The remaining sediment was collected into conical tubes and centrifuged at 3,000×g for 15 min to concentrate the parasitic stages. The supernatant was carefully collected after centrifugation, and the sediments were examined for parasite ova/cysts detection.

Detection of parasites

Four slides were prepared from each sample to increase

[DOI: 10.18502/jfqhc.10.1.11984]

the chance of detecting parasites. The first slide was inspected as unstained wet smear, whereas the second one was stained with Lugol's Iodine. Both slides were examined for parasitic protozoa and helminths using 10× and 40× objectives of a light microscope (Zeiss, Axio Scope.A1, Jena, Germany). On other slides, a thin smear of the sediment was applied, air dried, and then fixed for 3 min with methanol. The slides were stained with trichrome stain for detection of *Microsporidium* spp. spores, and modified Ziehl-Neelsen for detection of coccidian oocysts as *Cryptosporidium* spp., and *Cyclospora* spp. (WHO, 2019). Slides were then examined microscopically by oil immersion lens (100×). The microscopic examination was done 3 times on each slide by independent investigators for confirmation. Parasitic stages were recognized depending on their morphological characteristics according to WHO (2019).

Statistical analysis

Statistical software SPSS version 20 was used for data analysis. Calculation of the contamination rate percentage was done by dividing the number of positive samples on the number of total vegetables and the resulting number was multiplied by 100. Also, mean density (parasites/100 g of vegetables) was calculated. Chi-square test was used to compare the rate of parasite contamination in the studied vegetables, compare the vegetables contamination by a single parasite versus multiple parasites and also, compare the vegetables contamination by protozoa versus helminths. The *p* values<0.05 were considered as significant.

Results

Totally, 86 out of 100 studied vegetable samples were contaminated with different parasitic eggs, cysts, oocysts, and larvae (Figures 1 and 2). Coriander was the most contaminated vegetable with contamination rate of 95% (19 out of 20), while dill was the least contaminated (16 out of 20; 80%). Parsley, rocca, and mint gave the same contamination rates of 85% (17 out of 20). There was no significance difference between the rate of parasite contamination among five types of vegetables ($\chi2$=1.9934; *p*=0.736981).

Out of the overall contaminated samples, 36.1% had one type of parasite and the remaining 63.9% had multiple parasitic stages: double (41.86%), triple (16.27%), quadruple (4.65%), or quintuple (1.16%) parasites. There was no statistically significant difference even though the rate of vegetables contamination by multiple parasites was higher than rate of contamination by single parasite ($\chi2$=9.3527; *p*=0.052864; Table 1). Detected parasites included 128 (77.1%) protozoan cysts/oocysts, and 38 (22.9%) helminthic eggs, larvae, and worms. Although, the rate of contamination by protozoan parasites was higher than rate of contamination by helminth parasites, there was no significance difference ($\chi2$=5.3846; *p*=0.250063; Table 2).

Regarding the parasite prevalence among the studied vegetables, *E. histolytica/dispar* was the most encountered parasite with an estimated prevalence reaching 40.6% followed by *B. hominis* (39.5%), *A. lumbricoides* (18.6%), *Cyclospora* spp. (15.11%), *G. lamblia* (11.6%), and *E. coli* (9.3%). While *Cryptosporidium* spp., *I. bütschlii*, *T. trichiura*, *E. vermicularis*, *Fasciola*, and hookworms' eggs were the least prevalent (1.1%) parasites in the studied vegetables. Other detected parasites included *T. gondii*, *H. nana*, *S. stercoralis*, *E. nana*, *B. coli*, *C. mesinili*, *Microsporidia* spp., *H. diminuta*, and *Toxocara* spp. with prevalence rates ranging from 8.1 to 2.3% (Table 3).

Table 1: Intensity of parasitic contamination in studied vegetables samples from Giza, Egypt

Studied Vegetables	No. of contaminated samples	Single Contamination No. (%)	No. of mixed contamination				Total No. (%)
			Double cont.	Triple cont.	Quadruple cont.	Quintuple cont.	
Dill	16	6 (37.5)	6	3	1	0	10 (62.5)
Parsley	17	11 (64.7)	3	3	0	0	6 (35.3)
Coriander	19	5 (26.3)	13	1	0	0	14 (73.7)
Rocca	17	6 (35.3)	6	3	2	0	11 (64.7)
Mint	17	3 (17.6)	8	4	1	1	14 (82.4)
Total	86	31 (36.1)	36 (41.86%)	14 (16.27%)	4 (4.65%)	1 (1.16%)	55 (63.9)

$\chi2$=9.3527; *p*=0.052864

[DOI: 10.18502/jfqhc.10.1.11984]

Table 2: Prevalence of protozoan and helminths contamination in studied vegetables samples from Giza, Egypt

Studied Vegetables	Total No. of parasites	Protozoa Contamination No. (%)	Helminths Contamination No. (%)
Dill	31	26 (83.8)	5 (16.1)
Parsley	26	23 (88,5)	3 (11.5)
Coriander	34	26 (76.5)	8 (23.5)
Rocca	35	23 (65.7)	12 (34.3)
Mint	40	30 (75)	10 (25)
Total	166	128 (77.1)	38 (22.9)

$\chi 2$=5.3846; *p*=0.250063

Table 3: Distribution and prevalence of the detected parasites in contaminated vegetables samples from Giza, Egypt

Detected parasites	**No. of positive vegetables samples (86)**					**Total No.**	**Prevalence %**	**Mean density**
	Dill (16)	Parsley (17)	Coriander (19)	Rocca (17)	Mint (17)			
Protozoa								
Entamoeba histolytica/dispar cyst	6	8	6	5	10	35	40.6	7
Entamoeba coli cyst	1	2	2	3	0	8	9.3	1.6
Endolimax nana cyst	0	0	1	2	0	3	3.4	0.6
Iodamoeba bütschlii cyst	1	0	0	0	0	1	1.1	0.2
Chilomastix mesinili cyst	0	0	1	0	1	2	2.3	0.4
Balantidium coli cyst	0	0	0	2	1	3	3.4	0.6
Giardia. lamblia cyst	2	4	1	-	3	10	11.6	2
Cyclospora spp. oocyst	2	1	2	4	4	13	15.11	2.6
Cryptosporidium oocyst	0	0	0	0	1	1	1.1	0.2
Microsporidia spores	2	0	0	0	0	2	2.3	0.4
Blastocystis hominis cyst	9	6	9	7	3	34	39.5	6.8
Toxoplasma gondii oocyst	0	2	2	0	3	7	8.1	1.4
Unsporulated oocyst	3	0	2	0	4	9	10.4	1.8
Total No.	26	23	26	23	30	128		
Helminths								
Ascaris lumbricoides egg	1	1	3	8	3	16	18.6	3.2
Trichuris trichiura egg	0	0	0	0	1	1	1.1	0.2
Hymenolepis nana egg	1	1	0	1	3	6	6.9	1.2
Hymenolepis diminuta egg	1	0	1	0	0	2	2.3	0.4
Enterobius vermicularis egg	1	0	0	1	0	2	2.3	0.4
E. vermicularis worm	0	0	1	0	0	1	1.1	0.2
Strongyloides stercoralis larvae	0	1	2	2	1	6	6.9	1.2
Toxocara spp. egg	1	0	1	0	0	2	2.3	0.4
Fasciola egg	0	0	0	0	1	1	1.1	0.2
Hookworm egg	0	0	0	0	1	1	1.1	0.2
Total No.	5	3	8	12	10	38	9.6	3.2
Total No. of detected parasites							166	

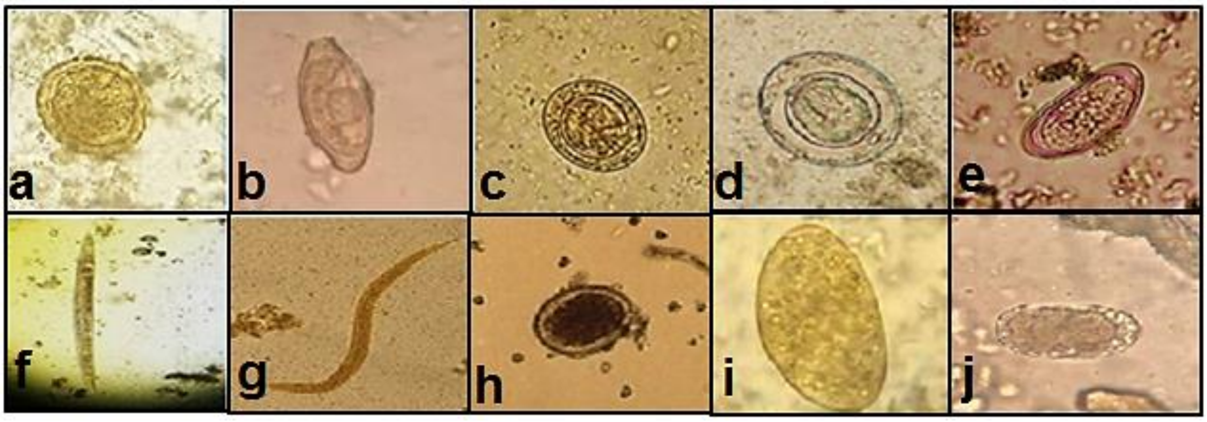

Figure 1: Detected helminthic eggs, larvae, and worms in the studied vegetables samples from Giza, Egypt: a: *Ascaris lumbricoides* egg; b: *Trichuris trichiura* egg; c: *Hymenolepis nana* egg; d: *Hymenolepis diminuta* egg; e: *Enterobius vermicularis* egg; f: *E. vermicularis* worm; g: *Strongyloides stercoralis* larva; h: *Toxocara* spp. egg; i: *Fasciola* egg; j: Hookworm egg

[DOI: 10.18502/jfqhc.10.1.11984]

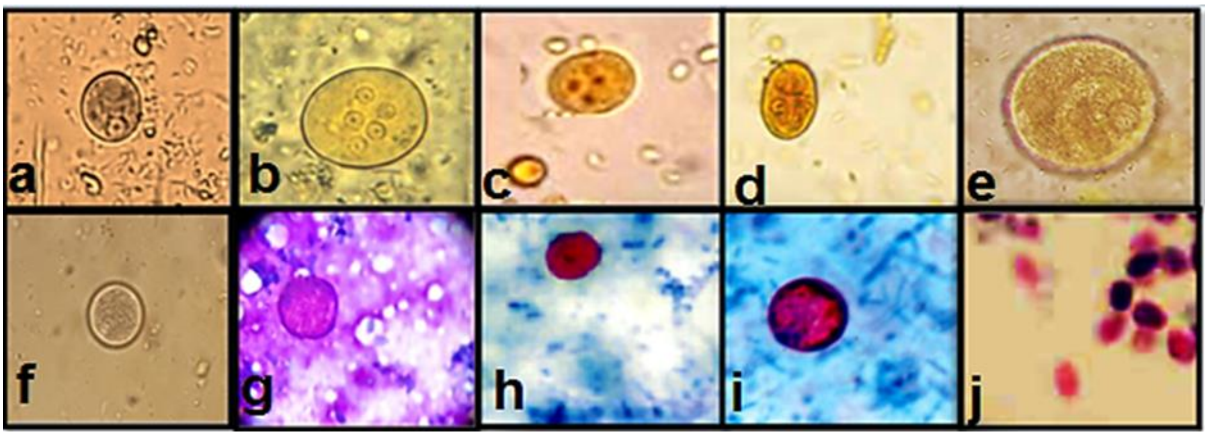

Figure 2: Detected protozoan cysts/oocysts in the studied vegetables samples from Giza, Egypt: a: *Entamoeba histolytica/dispar* cyst; b: *Entamoeba coli* cyst; c: *Endolimax nana* cyst; d: *Giardia lamblia* cyst; e: *Balantidium coli* cyst; f: *Toxoplasma gondii* oocyst; g: *Blastocystis hominis* cyst; h: *Cyclospora* spp. oocyst; i: *Cryptosporidium* spp. oocyst; j: *Microsporidia* spores

Discussion

Most Egyptians have a dietary practice of eating raw leafy vegetables such as dill, parsley, coriander, rocca, and mint with their meals to provide the body with a variety of nutrients and vitamins. The easier attachment of infective parasitic stages to the leaves of raw vegetables always raises the danger of vegetables-borne parasitic diseases (El-Sayed et al., 2021). Therefore, the current study was done to assess the level of contamination and the prevalence of various intestinal parasites in the most prevalent leafy vegetables consumed by Egyptians in Giza city.

The current study revealed a high level of contamination (86%) in the examined vegetables. This contamination could be due to the leaves' ability to retain parasites between them, as well as their irregular surfaces, which facilitate easier parasite attachment. Also, vegetable samples were gathered in the summer months when parasite dissemination and prevalence are often high, which contributed to the study's high contamination rate. Parasitic contamination of vegetables was documented in other Egyptian studies that were conducted in various cities. Amongst Egyptian cities, Assiut city had the highest contamination rate at 84.1% (Ahmed et al., 2020), while Alexandria city had the lowest contamination rate at 19.4% (Hassan et al., 2012). Moreover, our findings agreed with those of several researches conducted in various countries where the parasite contamination rate of vegetables ranged from a lower contamination rate of 15.1% (El Bakri et al., 2020) to a higher contamination rate of 100% (Al-Sanabani et al., 2016). This might be due to variations in the climatic conditions, investigated vegetables, and research methodologies between this study and other studies.

In this study, rate of contamination by protozoan parasites (77.1%) was higher than rate of contamination by helminth parasites (22.9%). This is in agreement with the findings of Elmajdoub et al. (2017), who found that the contamination rate was 79.4% for protozoan cysts and 47.6% for helminth parasites. The presence of multiple species of medically important parasites in the studied vegetables certainly reflects the high level of contamination and the persistence of intestinal parasitic infection in Giza city. This could result in widespread intestinal parasite infections among humans (Youssef and Uga, 2014). In the present study, *E. histolytica/dispar* has shown the highest percentage (40.6%) as it was detected in all studied vegetables. Such a high percentage was observed in a study from Nigeria in which *E. histolytica* was the only detected protozoan parasite (Oladele et al., 2022), however, the percentage was lower in Saudi Arabia report (3.1%; Al-Binali et al., 2006). Variations in geographic distribution and the cysts' ability to survive in cold, moist circumstances for a long time may be responsible for the predominance of *E. histolytica/dispar*.

B. hominis was the second most prevalent contaminant in the studied vegetables which recorded 39.5%. The obtained result agreed with the results of Al Nahhas and Aboualchamat (2020) in Syria (29.5%). However, Etewa et al. (2017) recorded a lower rate (3.8%) in Sharkyia, Egypt. The high prevalence of *B. hominis* might be attributed to the use of trichrome stain as a diagnostic tool in the present study in contrast to the other studies. Other parasites detected in this study included

[DOI: 10.18502/jfqhc.10.1.11984]

Cyclospora spp. (15.11%), *Microsporidia* (2.3%), and *Cryptosporidium* (1.1%) which are leading cause of diarrhea worldwide with significant morbidity and mortality particularly in the immune-compromised individuals. However, much higher incidence rate of these parasites reported in another Egyptian study by Said (2012) who recorded *Cryptosporidium* oocysts in 29.3% of raw vegetables followed by *Microsporidia* (25.3%) and *Cyclospora* (21.3%). The difference between this study and others might be due to differences in the studied vegetables, sample size, and used methods.

Result of this research, showing 11.6% of vegetables were *G. lamblia*-contaminated, is consistent with previous research in Egypt, done in Alexandria, by Said (2012) and Hassan et al. (2020) who reported prevalence's rates of 6.7 and 8.8%, respectively. Similar finding was drawn from Libyan research, where 10% of all examined vegetable samples had *G. lamblia* cysts (Abougrain et al., 2010). Furthermore, this result is compatible with the percentage of Egyptians infected with giardiasis, which ranges from 10 to 34.6% (Youssef and Uga, 2014). This prevalence may be attributed to the *Giardia*'s ability to survive under cool and moist conditions as well as its resistance to chlorine in drinking water. *B. coli* was also found in 3.4% of the tested vegetables. However, it was the most prevalent parasite contaminant in the studies conducted in Cameroon (25.6%; Akoachere et al., 2018) and Ghana (13.6%; Kudah et al., 2018). *B. coli*-contaminated vegetables may be caused by contaminating irrigation water with humans and pigs feces (Kudah et al., 2018). Other detected intestinal protozoa in this study were *E. coli* (9.3%), *E. nana* (3.4%), *I. bütschlii* (1.1%), and *C. mesinili* (2.3%). The presence of these non-pathogenic parasites in vegetable samples indicated a low level of sanitation, as the vegetables were contaminated with human feces.

We found that 8.1% of vegetables were contaminated with *T. gondii* oocysts due to substantial contamination of the Egyptian environment by stray cats feces. This result agrees with that found by Marček et al. (2018) where 6.7% of examined lettuce was contaminated with *T. gondii* oocysts. However, other studies relied on the detection of *Toxoplasma* DNA on the examined vegetables which certainly will give much higher contamination rates (Marques et al., 2020). Toxoplasmosis is considered a significant health problem among Egyptian population with high sero-prevalence levels ranging from 33.67% (El-Sayed et al., 2016a) to 54% (El-Sayed et al., 2016b).

Vegetables that are frequently fertilized with natural fertilizers such as animal excreta are more liable to contamination. *A. lumbricoides*, *Toxocara* spp., *T. trichiura*, and *Ancylostoma duodenale* are among the parasites whose eggs are present in this manure. Over time, these eggs will mature and eventually reach infective stages, which might lead to helminth infections. Regarding helminthic infections detected in this study, the parasite with the highest prevalence was *A. lumbricoides* (18.6%). This might be because the female parasite producing a large number of eggs that are resistant to harsh environments (Roberts, 2009) and also, the embryonated egg's albuminoid coat characteristic that allows the parasite to adhere easily to vegetables. The prevalence of *A. lumbricoides* is consistent with findings from earlier studies in Egypt (20.3%; Said, 2012), Ethiopia (20.8%; Bekele et al., 2017), and Pakistan (26.7%; Khan et al., 2017). Although, prevalence of hookworm and *T. trichiura* seems to be low (1.1%) in this research, it could indicate fecal pollution of the environment and inadequate sewage disposal system. Detection of *Toxocara* eggs in the studied vegetables with a rate of 2.3% might be related to the substantial contamination of the Egyptian environment by stray dogs and cats feces as well as the eggs' resistance to extreme environmental conditions, which may survive for up to ten years. The prevalence rate of *Toxocara* eggs in this study (2.3%) was consistent with what reported by Punsawad et al. (2019) in Thailand (2.6%). Many researches have documented the high prevalence of human toxocariasis, either visceral (El-Sayed and Ramadan, 2017b) or ocular (El-Sayed and Masoud, 2019) in Egypt.

H. nana was the second most frequent helminthic contamination in the studied vegetables, accounting for 6.9%. This percentage was comparable to that recorded in Libya (7.9%) (Elmajdoub et al., 2017). On contrast, other studies showed higher rate in Ethiopia (15.56%; Bekele et al., 2017) and lower rate as detected in Qazvin, Iran, (0.5%; Shahnazi and Jafari-Sabet, 2010). Differences in the environmental conditions and also geographical location could explain the observed disparity. According to numerous researchers, hymenolypiasis infections are exceedingly common in Egyptian patients, with high detection rates (Youssef and Uga, 2014).

Strongyloides larvae were detected at a prevalence of 6.9% in this study, which could be attributable to the parasite's free-living state as well as its parasitic lifestyle. The preponderance of *Strongyloides* is consistent with findings from earlier studies (Al Nahhas and Aboualchamat, 2020; Alemu et al., 2019; Elmajdoub et al., 2017; Mohamed et al., 2016; Punsawad et al., 2019). However, *S. stercoralis* may not pose a considerable risk to consumers because human infection is primarily transmitted to barefoot individuals through larval stages penetrating their skin rather than through eating.

According to Egyptian studies, fascioliasis seems to be hyperendemic in Nile Delta villages with the mean

[DOI: 10.18502/jfqhc.10.1.11984]

prevalence of 12.8%, the highest level ever recorded in Egypt (Esteban et al., 2003). However, just 1.1% of the vegetable samples in this study were contaminated with *Fasciola* eggs. Similarly, Fawzi et al. (2004) detected *Fasciola* eggs in 2.4% of the vegetable samples from a village in Alexandria governorate, Egypt. Also, other detected helminthic contaminations in the studied vegetables were *H. diminuta* and *E. vermicularis* with a same prevalence rate of 2.3%. This ensures that vegetables are fertilized with untreated human and animal wastes. In a study in Philippines, *H. diminuta* was reported to be 1% (Vizon et al., 2019) and in another study in Southwest Ethiopia, it was reported 1.4% (Tefera et al., 2014). But the results are different from those of other researchers in Iraq (4%; Saida and Nooraldeen, 2014) and southern Ethiopia (7.7%; Bekele et al., 2017). Regarding *E. vermicularis*, Etewa et al. (2017) in Sharkyia, Egypt reported 1.4% while El Bakri et al. (2020) in United Arab Emirates, reported higher rate of 6.1%. The differences between our study and prior studies may be due to differences in geographic regions, climatological or environmental variables, use of wastewater for irrigating vegetables, sample type and size, sampling processes, intestinal parasite detection methods, cross contamination during unhygienic transportation or market handling, and socioeconomic status (Said, 2012). As long as these, variables are different, there will be a disparity in the observed results.

Conclusion

The results of this study showed that leafy vegetables had a very high parasite contamination rate in Giza, Egypt. Eating of such vegetables in raw state facilitates transmission of intestinal parasitic infections that may cause a major health risk to Egyptians. To avoid risk of vegetables-borne parasitic diseases, additional research should be done to determine the degree of contamination of the soil and irrigation water used to grow these vegetables. Nevertheless, preventing contamination continues to be the most effective strategy to reduce these diseases. Both environmental and health organizations should collaborate to improve the sanitary conditions for vegetables cultivation and consumption. Consumers should be informed about the health concerns of consumption of contaminated vegetables through media programs, as well as the need to wash and disinfect vegetables before eating.

Author contributions

N.M.E.-S proposed the research idea and the study design, collected the study samples, shared in laboratory performance of the work, interpreted the results, wrote the manuscript; S.S.G. collected the study samples, assisted in performing the laboratory work, and revised the manuscript; H.S.E.-K. and A.E. assisted in performing the laboratory work. Each author examined carefully the prepared slides separately and all agreed on the positivity of samples by microscopy. All authors read and approved the final manuscript.

Conflicts of interest

None of the authors have conflicts.

Acknowledgements

This study did not receive any specific grant from funding agencies in the public, commercial, or non -for-profit sectors.

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