Alexandria Journal of Medicine (2012) 48, 345–352

Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

www.sciencedirect.com

ORIGINAL ARTICLE

Detection of parasites in commonly consumed raw vegetables

Doaa El Said Said

Alexandria University, Faculty of Medicine, Alexandria, Egypt

Received 8 April 2012; accepted 28 May 2012
Available online 25 September 2012

KEYWORDS
Green vegetables;
Parasites;
Seasonal variations;
Alexandria;
Egypt

Abstract *Background:* Ingestion of raw vegetables represented an important mean of transmission of several infectious diseases. No previous surveys have been conducted to evaluate the prevalence of parasitic contamination of vegetables in Egypt. Therefore, this study aimed to detect the parasitic contamination in some common raw vegetables in Alexandria, Egypt.
Methods: It was based on: extraction of parasitic stages from the foodstuffs, concentration of the extract by centrifugation, staining of the different stages by modified Zeihl–Neelsen stain and modified trichrome stain to allow visualization of protozoal oocysts and spores of *Microsporidium*, and identification by microscopy. Scores of parasite density were evaluated.
Results: Intestinal parasites were detected in 31.7% of the examined samples. The parasites included *Ascaris lumbricoides* eggs, *Toxocara* spp. eggs, *Hymenolepis nana* eggs, *Giardia* spp. cysts, *Cryptosporidium* spp. oocysts, *Cyclospora* spp. oocysts and *Microsporidium* spp. spores. The highest number of contaminated samples was detected in rocket (46.7%) while the least number of contaminated samples was detected in green onion (13.3%). *Cryptosporidium* spp. oocysts were considered the highest prevalent parasite detected in raw vegetables (29.3%) with the highest score density in rocket samples. It was followed by *Microsporidium* spp. spores (25.3%), *Cyclospora* spp. oocysts (21.3%), *A. lumbricoides* eggs (20.3%), *Toxocara* spp. eggs (19%), *Giardia* spp. cysts (6.7%) and finally *H. nana* eggs (2.6%). There was no significant difference between the number of contaminated samples in spring and summer or autumn and winter. However, the number was significantly higher in spring and summer in comparison to winter and autumn ($p < 0.001$).
Conclusion: These findings may have important implications for global food safety and emphasize the importance of raw vegetables in threatening public health by transmission of intestinal parasites to humans in Alexandria, Egypt.

E-mail address: drdoaa_1995@hotmail.com
Peer review under responsibility of Alexandria University Faculty of Medicine.

ELSEVIER Production and hosting by Elsevier

1. Introduction

Vegetables are essential part of a healthy human diet owing to their nutritional value. Raw vegetables are great source of vitamins, dietary fiber and minerals; and their regular consumption is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers.[1] Some vegetables are eaten raw as

2090-5068
http://dx.doi.org/10.1016/j.ajme.2012.05.005

salad to retain the natural taste and preserve heat labile nutrients. Ingestion of raw vegetables represented an important mean of transmission of several infectious diseases because of their complex surface and porosity, which unfortunately facilitate pathogen attachment and survival.[2] The consumption of raw vegetables without proper washing is an important route in the transmission of parasitic diseases.[3] There has been an increase in the number of reported cases of food-borne illness linked to fresh vegetables. Several factors may contribute to contamination of crops. They become contaminated while still on the plant in fields, Orchards, or during harvesting, transport, processing, distribution and marketing or even at home.[4] Wastewater was increasingly used for irrigation in the 1970s and early 1980s. In many developing countries, use of insufficiently treated wastewater to irrigate vegetables has been reported to be responsible for the high rates of contamination with pathogenic parasites.[4] Contamination of soil with animal wastes and increased application of improperly composted manures to soil in which vegetables are grown also play a role in parasite contamination to green vegetables.[5] Bad hygienic practice during production, transport, processing and preparation by handlers including consumers also contribute in vegetable contaminations.[6] Other factors which affect the susceptibility of the public to foodborne diseases, also play a role in increasing the number of infected cases. Highly susceptible persons because of ageing, malnutrition, HIV infection and other underlying medical conditions are markedly increased. Changes in lifestyle, food consumption patterns, such as the increase in the number of people eating meals prepared in restaurants, canteens and fast food outlets as well as from street food vendors who do not always respect food safety increase the risk of exposure to foodborne infections.[7]

In the past, the risk of human infection with parasites was considered to be limited to distinct geographic regions because of parasites' adaptations to specific definitive hosts, selective intermediate hosts and particular environmental conditions. These barriers are slowly being breached—first by international travel developing into a major industry, and second, by rapid, refrigerated food transport which became available to an unprecedented degree at the end of the 20th century. Taking into account that a proportion of the vegetables cultivated in these developing countries are exported to developed ones, the risk of spreading these contaminations to other countries cannot be overlooked.[8]

In developing countries, because of inadequate or even non-existing systems for routine diagnosis and monitoring or reporting for many of the food-borne pathogens, most outbreaks caused by contaminated vegetables go undetected and the incidence of their occurrence in food is underestimated.[9] Practical and reliable detection methods for monitoring foodstuffs will aid the prevention of parasitic disease outbreaks associated with contaminated food.[10] Referring to existing scientific literatures, no previous surveys have been conducted to evaluate the presence of parasitic contamination in vegetables in Egypt. Therefore, this study aimed to detect the parasitic contamination in some common green vegetables used for raw consumption in Alexandria, Egypt.

2. Material and methods

2.1. Sample collection

Five types of green vegetables were selected in this study {lettuce (*Lactuca sativa*), rocket (*Eruca sativa*), parsley (*Petroselinum hortense*), leek (*Allium porrum*) and green onion (*Allium ascalonicum*)}. A total of 300 samples (60 samples from each type of green vegetables) were purchased at the smallest retail size available. Samples were collected monthly (five sample for each type of green vegetables) and were picked randomly from commercial groceries, supermarkets, street vendors, and food stall markets in Alexandria, Egypt during May 2010–April 2011. Two hundred grams were being prepared from the edible part of each vegetable sample separately according to the household practice to obtain qualitative and quantitative estimation of parasitic contamination.

2.2. Procedure for sample preparation and washing

The vegetable samples were transported to laboratory in plastic bags. According to the traditional procedure which is generally used for washing vegetables, they were immersed immediately in tap water inside a sink and left approximately 6–7 min for sedimentation of mud and dust. Then, it was gently collected and was put in a plastic basket. Each vegetable sample was eluted by vigorous agitation followed by sonication (INC. ultrasonic processor W-380, Japan) of each specimen for 30 min in 1 L of sterile phosphate-buffered saline (pH 7.4), to which 50 ml of 0.01% Tween 80 was added. The eluent was filtered through gauze and then dispensed into clean centrifuge tubes and centrifuged at 2000×*g* for 30 min. The supernatant was discarded into disinfectant jar, and the pellet was washed of Tween 80 by centrifugation (2000×*g* for 30 min) with sterile phosphate-buffered saline in the standard procedure described by Gaspard and Schwartzbrod.[11]

2.3. Detection of parasites

The sediment was mixed and examined as follow:

(1) Simple smear: a drop of the sediment was applied on the center of a clean grease-free slide. A clean cover slip was placed gently to avoid air bubbles and over flooding. The preparation was examined under a light microscope using ×10 and ×40 objectives.[12]
(2) Iodine smear: a drop of the sediment was mixed with a drop of Lugol's Iodine solution and examined as in simple smear.[12]
(3) Simple and iodine smears were used for detection of parasitic eggs, cysts and larva. The process was systematically repeated until the mixture in each test tube was exhausted. Eggs, cysts and oocysts of parasites found under the light microscope were identified as previously described by Downes and Ito.[13]
(4) Staining of sediment smear was performed by Modified Zeihl–Neelsen[14] and modified trichrome[15] to detect protozoal parasitic (oo)cysts and spores of *Microsporidium* spp.

Each parasite's eggs, cysts or oocysts present in each sample were enumerated and densities of each species were expressed as "many" (> three (oo) cysts per high-power field; > 20 eggs per low-power field); "moderate" (two (oo)cysts per high-power field; 10–19 eggs per-low power field); "few" (one (oo)-cyst per high-power field; three to nine eggs per low-power field); and "rare" (two to five cysts and < two eggs per 100μ of sediment). For simplification, numerical values were assigned to each density: many, 4; moderate, 3; few, 2; rare, 1; and none, 0.[16]

2.4. The statistical analysis

Statistical analyses were carried out using chi-square tests of the SPSS software version 9.0 for windows (SPSS Inc., Chicago, IL, USA) to compare the rate of contamination of vegetables among different seasons. The differences were considered significant at $p < 0.05$.

3. Results

Results are summarized in Tables 1 and 2 and Figs. 1 and 2.

Intestinal parasites were detected in 31.7% of the examined samples (Fig. 1). The parasites detected in raw green vegetable samples were *Cryptosporidium* spp. oocysts, *Microsporidium* spp. spores, *Cyclospora* spp. oocysts, *Giardia* spp. cysts *Ascaris lumbricoides* eggs, *Toxocara* spp. eggs, and *Hymenolepis nana* eggs. The highest number of contaminated samples was detected in rocket (46.7%) while the least number of contaminated samples was detected in green onion (13.3%) (Table 1 and Fig. 2).

Rocket was the most contaminated green vegetable in this study (46.7%). *Cryptosporidium* spp. were the most prevalent parasite in rocket samples (45%). *Microsporidium* spp. spores contamination followed that of *Cryptosporidium* spp. oocysts (36.7%). This was followed by *Ascaris* eggs (35%), *Cyclospora* spp. oocysts (31.7%), *Toxocara eggs* (25%), *Giardia* spp. cysts (10%) and finally *H. nana* eggs (3.3%) (Fig. 2 and Table 1).

As regards lettuce samples, 45% were contaminated with parasites. The most prevalent parasite detected in lettuce was *Cryptosporidium* spp. oocysts (43.3%) followed by *Microsporidium* spp. spores (41.7%). The number of contaminated lettuce samples with *Ascaris* eggs and *Cyclospora* spp. oocysts was equal (38.3%). This was followed by *Toxocara eggs* (31.7%), *Giardia* spp. cysts (15%) and finally *H. nana* eggs (6.7%) (Fig. 2 and Table 1).

Contamination of parsley samples was detected in 36.7% of the total samples. They were mainly contaminated with *Cryptosporidium* spp. oocysts (33.3%). This was followed by *Cyclospora* spp. oocysts (25%), and *Toxocara* eggs (20%). The number of contaminated parsley samples with *Microsporidium* spp. spores and *Ascaris* eggs is equal (16.7%). The least number of contaminated samples was detected in samples contaminated with *Giardia* spp. cysts and *H. nana* eggs (5% and 3.3% respectively) (Fig. 2 and Table 1).

Regarding leek contaminated samples (16.7%), they were mainly contaminated with *Cryptosporidium* spp. oocysts (14.3%). *Cyclospora* spp. oocysts, and *Toxocara* eggs contaminated samples were equal (8.3%). *Ascaris* eggs and *Giardia* spp. cysts were detected in 6.7% and 1.7% of parsley samples respectively. *H. nana* eggs were not detected in any of parsley sample (0%). (Fig. 2 and Table 1).

Green onion samples were the least contaminated samples in this study. Contamination was detected in only 13.3% of the examined samples. The highest contamination was by *Microsporidia* spp. (18.3%) followed by *Cryptosporidium* spp. oocysts (11.7%), *Toxocara* eggs in 10%, *Ascaris* eggs in 5% and finally *Cyclospora* spp. oocysts in 3.3%. *H. nana* eggs and *Giardia* spp. cysts were not detected in any of green onion samples (0%) (Fig. 2 and Table 1).

Cryptosporidium spp. oocysts were considered the highest prevalent parasite detected in raw vegetables (29.3%). It was followed by *Microsporidium* spp. spores in 25.3%, *Cyclospora* spp. oocysts in 21.3%, *A. lumbricoides* eggs in 20.3%, *Toxocara* spp. Eggs in 19%, *Giardia* spp. cysts in 6.7% and finally *H. nana* eggs in 2.6% (Table 1).

The mean of parasite score density in the contaminated samples ranged between (2.22) and (0). The highest score of parasite density was that of *Cryptosporidium* spp. oocysts (2.22). It was detected in contaminated rocket samples. However, the least score of parasite density was that of *H. nana* eggs (0) in parsley, leek and green onion samples. The least score was also detected in green onion samples with *Giardia* spp. cysts (0) (Table 1). The highest rate of parasitic contamination in vegetable samples in different seasons was found in spring (49.3%), followed by summer (48%). There was no significant difference between the number of contaminated samples in these two seasons. Lesser contamination rates were found in autumn (20%) and in winter (9.3%). There was also no significant difference between these two seasons. Comparing the rate of parasitic contamination in the four seasons, it was found that the number of contaminated samples in spring was significantly higher than the number of contaminated samples in winter and autumn. Also the number of contaminated samples in summer was significantly higher when compared to the number of contaminated samples in winter and autumn ($x^2 = 42.069$; $p < 0.001$). The highest rate of contamination in spring was found in lettuce samples (73.3%) while the lowest contamination in the same season was in leek (20%). In winter, the number of contaminated samples was equal in rocket, parsley and leek (13.3%). One sample only was contaminated in lettuce (6.6%) however no parasite was detected in green onion samples during this season (0%) (Table 2).

4. Discussion

Different parasitic stages can contaminate several foodstuffs. If the food-borne route is suspected in an outbreak, it is easy to identify how the food implicated became contaminated. However, it is often difficult to associate an outbreak with a particular food item.[3] Vegetables, and especially salads, are an important route of transmission of intestinal parasites and have been shown to be an important source of food borne outbreaks in developing countries.[17] The most likely hypothesis of contamination is that it occurred before harvest, either by contaminated manure, manure compost, sewage sludge, irrigation water, runoff water from livestock operations or directly from wild and domestic animals. These potential contamination events are all plausible and consistent with the assumption that the level of contamination must have been high.[18]

Alexandria is a large coastal city. It is considered the second capital of Egypt after Cairo. It is mainly an industrial city, with some agriculture fields.[19] Therefore, the present study aimed at investigating some green vegetables that are frequently eaten raw for their possible contamination with parasites in Alexandria. This study showed a considerably high level of contamination of green vegetables foodstuff with intestinal parasites in Alexandria (31.7%). These results were in agreement with

Table 1 The number and percentage of contaminated samples and the mean of parasite score density in some raw green vegetables.

	Rocket		Lettuce		Parsley		Leek		Green onion		Total No. (%) $n2 = 300$
	No. (%) $n1 = 60$	*D*	No. (%) $n1 = 60$	*D*	No. (%) $n1 = 60$	*D*	No. (%) $n1 = 60$	*D*	No. (%) $n1 = 60$	D	
Cryptosporidia spp. oocysts	27(45)	2.22	26 (43.3)	2.10	20 (33.3)	1.90	8 (13.3)	1.10	7 (11.7)	1.30	88 (29.3)
Microsporidia spp. spores	22 (36.7)	1.80	25 (41.7)	2.00	10 (16.7)	1.80	8 (13.3)	1.42	11(18.3)	1.34	76 (25.3)
Cyclospora spp. oocysts	19 (31.7)	1.88	23 (38.3)	1.95	15 (25)	1.74	5 (8.3)	1.60	2 (3.3)	1.50	64 (21.3)
Ascaris eggs	21(35%)	1.30	23 (38.3)	1.75	10 (16.7)	1.42	4 (6.7)	1.25	3(5)	1.33	61 (20.3)
Toxocara eggs	15 (25)	1.86	19(31.7)	1.81	12 (20)	1.54	5 (8.3)	1.02	6(10)	1.00	57 (19)
Giardia spp. cysts	6 (10)	1.00	9 (15)	0.88	3 (5)	0.66	1(1.7)	1.00	0 (0)	0	19 (6.7)
Hymenolepis nana eggs	2(3.3)	0.33	4 (6.7)	0.50	2 (3.3)	0.03	0 (0)	0	0 (0)	0	8 (2.6)

No.: Number of contaminated sample by each parasite.
$n1$ = Number of examined samples for each type of green vegetable = 60.
D: mean score density for each parasite in green vegetables.
Total No.: Total number of contaminated samples by each parasite.
$n2$: Total number of examined samples = 300.

Table 2 The number and percentage of contaminated samples with intestinal parasites in different seasons in some raw green vegetables.

Season	Lettuce No. (%) $n1 = 15$	Rocket No. (%) $n1 = 15$	Parsley No. (%) $n1 = 15$	Leek No. (%) $n1 = 15$	Green onion No. (%) $n1 = 15$	Total No. %/season $n2 = 75$
Spring	11 (73.3)	10 (66.7)	9 (60)	3 (20)	4 (26.7)	37 (49.3)
Summer	12 (80)	10 (66.7)	8 (53.3)	3 (20)	3 (20)	36 (48)
Autumn	3 (20)	6 (40)	3 (20)	2 (13.3)	1 (6.7)	15 (20)
Winter	1 (6.7)	2 (13.3)	2 (13.3)	2 (13.3)	0 (0)	7 (9.3)

No.: Number of contaminated samples in each type of green vegetables/season.
$n1$ = Total number of examined samples for each type of green vegetable/season = 15.
Total No./season: Total number of contaminated samples in each type of raw vegetables/season.
$n2$: Total number of examined samples of green vegetables/season = 75.
($x^2 = 42.069$), p value considered significant at $p < 0.05$.

another one in Ghana which evaluated the overall parasitic contamination of the vegetables as 36%.[20] Parasitic contamination of the vegetables in another study in Nigeria was also 36%.[21] Recently, Ettehad et al.[22] reported, slightly lower level of contamination of consumed native garden vegetables with intestinal parasites (29%) in Ardabil city, Iran. Higher rate of contamination of green vegetables was detected in wholesale and retail markets in Tripoli, Libya in the study carried out by Abougraina in 2009. This study detected 58% positive samples for intestinal parasites.[23] Examination of vegetable samples in Kenya by Nyarango in 2008 revealed also higher rate of contamination (75.9%).[16]

In the present work, five types of common consumed raw vegetables in Alexandria were examined; rocket, lettuce, parsley, leek and green onion. The highest rate of contamination was detected in rocket samples (46.7%). It was followed by lettuce samples (45%). This could be due to the fact that the degree of contamination varies according to the shape and surface of vegetables. Green leafy vegetables as lettuce, rocket and parsley have uneven surfaces and makes parasitic eggs, cysts and oocysts attached to the surface of the vegetable more easily, either in the farm or when washed with contaminated water. On the other hand, vegetables with smooth surface as leek and green onion had the least prevalence because its smooth surface reduces the rate of parasitic attachment.[21]

These results were in agreement with that of a study done in Tripoli, Libya. It detected parasitic contamination in 96% of lettuce and 100% of cress samples. Lettuce and cress samples were contaminated significantly more often than those of tomato samples in the same study.[23] Damen et al.,[21] detected contamination in 40% of lettuce samples, and 24% in green leafy vegetable in Nigeria. In contrast, other study in Saudi Arabia in 2006 revealed that the highest number of contaminated samples was found in green onions (28%) and the lowest contamination was in leek (13%). The prevalence of parasites in other vegetables in the same study was 17% in each of lettuce and water cress.[24] Another two studies in Iran reported that the highest contamination was in leek (51.8% and 66.7%).[25,26] Several factors may contribute to such differences. These may include, geographical location, type and number of samples examined, methods used for detection of the intestinal parasites, type of water used for irrigation, and post-harvesting handling methods of such vegetables which are different from country to another.

In the present work, *Cryptosporidium* was the most prevalent parasite contaminating green vegetable samples (29.3%). A recent paper by researchers in USA demonstrated that *Cryptosporidium* oocysts were capable of strongly adhering to spinach plants after contact with contaminated water and were also internalized within the leaves.[27] Although contamination of vegetables may occur in a variety of ways, it is mainly associated with the water used for irrigation. The use of sewage contaminated water for irrigation of vegetables is a common practice in developing countries including Egypt. The main water sources used for irrigation of fields in Alexandria are river water from El Mahmoudeya canal, untreated wastewater and rarely rain water.[28] A study done in Alexandria on detection of *Cryptosporidium* oocysts in irrigation water from El Mahmoudeya canal detected a high rate of contamination of irrigation water with this parasite (100%).[29] Specifically, *Cryptosporidium* was linked to the consumption of vegetables in Nordic countries.[30,31] In addition, *Cryptosporidium* was found in 4% samples from fresh produce in the study carried out by Robertson and Gjerde in Norway in 2001.[32] These also agree with another study in Costa Rica which reports the occurrence of some pathogenic micro-organisms in vegetables consumed on a daily basis. In this study, *Cryptosporidium* spp. oocysts were found in 2.5% of lettuce samples. In addition, it was reported in 5.2% of cilantro leaves and in 8.7% of cilantro roots. A 1.2% incidence was found in other vegetable samples (carrot, cucumber, radish and tomatoes). Oocysts of this parasite were absent, in the same study, in cabbage.[33] On the other hand, out of 19 salad products in a field study in Valencia, Spain, *Cryptosporidium* oocysts were detected in 12 samples (63.2%).[34]

Microsporidian spores are the second prevalent parasites in green vegetables in this study (25.3%). The highest contamination was detected in lettuce samples (41.7%). This may be due to the strong adhesion to, or internalization in leafy vegetables, thereby successfully evading the effects of washing and disinfection.[35] *Microsporidian* spores resist standard wastewater treatment and can be found in sewage sludge end products commonly used for fertilization of ready-to-eat crops or in runoff-impacted surface water used for irrigation.[36] *Microsporidian* spores have also been identified in irrigation water used for ready-to-eat crop production.[37] Reports on contamination of green vegetables with *Microsporidian* spores are scanty. Unspecified spore species have been found in samples from strawberries, lettuce, and parsley in the study carried out by Calvo in 2004.[33] A study was done in Sweden suggesting that cucumber slices in both cheese sandwiches and a salad were the most probable vehicle of transmission of *Microsporidian* spores causing an outbreak.[35] Several studies also detected spores in ground,[38] surface water[38,39] and crop-irrigation water,[37] and in fresh produce such as raspberries, beans and lettuce.[40]

Cyclospora oocysts in the present study were detected in 21.3% of the examined samples. Lettuce samples were also the main type contaminated with *Cyclospora* oocysts (38.3%). This parasite has also been isolated from lettuce in Peru (1.8%).[41] It was also isolated from lettuce heads in Egypt.[42] Green leafy vegetables in another study in Nepal were contaminated with *Cyclospora* oocysts.[43] These reports also agree with a study in Germany in which the only foods associated with *Cyclospora* risk of infection were two salad side dishes prepared from lettuce imported from southern Europe and spiced with fresh green leafy herbs.[44] Large-scale outbreaks of *Cyclospora* spp. infection associated with raspberries imported from Guatemala, basil, and mesclun lettuce have been also reported.[44–46]

In the present study, *Ascaris* eggs were detected in 20.3% of the examined samples. The highest contamination was detected in lettuce samples (38.3%). In a study done in India, *Ascaris* eggs were the most predominant parasite observed. It was detected in 36% of the examined samples, mainly in lettuce.[47] The rates of contamination with *Ascaris* eggs in vegetables in Iran were much lower than these values. It was 2.5% in Jiruft[48] and 2.3% in Qazvin.[26] The rate of contamination in Saudi Arabia was also lower than that detected in this study. It was 16% in leafy vegetables examined.[24] A study in Tripoli, Libya reported a higher rate of contamination (68%) of fresh salad vegetables with *Ascaris* eggs. They were detected in tomato (19%), cucumber (75%), lettuce (96%) and cress (96%), respectively.[23] In Ghana, Amoah, (2006) identified *Ascaris eggs* in 60% of the vegetables obtained from urban markets. This could be attributed to its high level of persistence and resistance. The presence of *Ascaris* eggs in vegetables can be due to the quality of water used for irrigation and the probable use of untreated night soil.[20] In Sanliurfa, Turkey, soil-transmitted helminthes (mainly *Ascaris* eggs) were detected in 14% of fresh vegetables and in 84% of soil samples where vegetables were cultivated in 61% of irrigation water.[49] Two previous studies in Alexandria detected *Ascaris* eggs in 1.9% and 0.16% of soil samples collected from different areas.[50,51] Biologically, the highest health risk is for helminth infections compared with other pathogens because helminthes persist for longer periods in the environment and the infective dose is small.[52]

In the present work, *Toxocara* eggs were detected in19% of the examined samples. Lettuce samples were the main vegetable contaminated with this parasite (31.7%). In Tripoli, Libya, *Toxocara* eggs were detected in 11%, 14%, 48% and 41% of the tomato, cucumber, lettuce, and cress samples respectively.[23] Kozan et al. in Turkey in 2007,[53] detected *Toxocara* spp. in 1.5% of unwashed raw vegetables used for salad. *Toxocara* spp. were detected in another study carried out in Turkey by Avcioglu in 2011 in four (2.0%) and two (1.0%) unwashed vegetables, respectively, mostly among leafy vegetables such as lettuce and parsley.[54]

The prevalence of *Giardia* spp. cysts detected in this study was 6.7%. The highest contamination was detected in lettuce (in 15% of the examined samples). The finding of this study is similar to previous reports in Iran. It was 8.2% in Shahrekord,[55] 14% in Jiruft,[48] 6.5% in Tehran,[56] 9% in Ardabil[22] and 4% in Qazvin.[57] In Saudi Arabia, in the study carried out by Al-Megrin 2010, 31.6% of leafy vegetables were contaminated with *Giardia* spp. cysts.[58] Robertson and Gjerde[32] reported a contamination rate of 2.1% with *Giardia* spp. in fruits and vegetables in Norway. *Giardia intestinalis* was only detected in 5.2% of cilantro leaves and in 2.5% of cilantro roots in Costa Rica.[33] Erdogru and Sener[59] found *Giardia* cysts in only 5.5% of lettuce samples. Contrary to these findings, another study in Libya detected *Giardia* cysts in 10% of the total vegetable samples examined with cucumber samples being most contaminated (19%), followed by cress (11%), lettuce (4%) and tomato (3%) samples.[23]

H. nana eggs were the least parasite contaminating green vegetables in this study. They were detected in 2.6% of the examined samples. Lettuce was the main type of vegetable contaminated with this parasite (6.7%). They were not detected in leek or green onion samples. *H. nana* eggs were detected in a previous study in Libya, in 2.4% of the total examined samples. In this study, cabbage had the highest parasitic contamination (64%) and tomatoes recorded the least parasitic contamination (20%).[23] In another study in Turkey, *H. nana* eggs were detected in 6.25% of examined samples.[53] In Qazvin, Iran, *H. nana* eggs were detected in 0.5% only of tested sam-

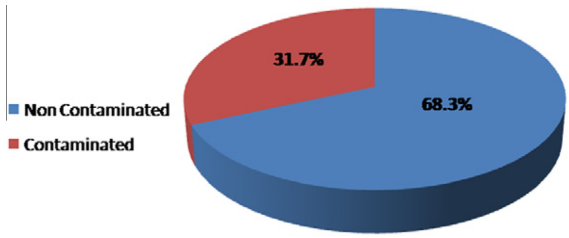

Figure 1 Percentage of the total number of contaminated and non contaminated samples of green vegetables in Alexandria, Egypt.

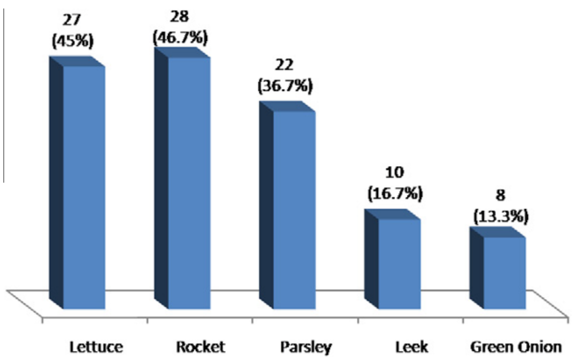

Figure 2 The number and percentage of contaminated samples in each type of green vegetable in Alexandria, Egypt.

ples and it was the least parasite contaminating the green vegetables. Leek was the highest contaminated vegetable with this parasite in the same study (66.7%).[57]

The presence of helminth eggs in different vegetables is mainly related to contamination of soil rather than contamination of irrigating water.[49] In a previous study on soil transmitted parasites in Alexandria, *Toxocara eggs* and *H. nana* eggs were detected in 16.7% in El-Ameria. *H. nana* eggs were also detected in Sharek district while, *Toxocara eggs* were also detected in Sharek, El-Montazah, Waset, Gharb, El-Gomrok and Borg El-Arab.[50]

Considering seasonal variability, this study indicated that the rate of parasitic contamination in vegetable samples was the highest in spring (49.3%) and the lowest in winter (9.3%). However there was no significant difference between spring and summer and between autumn and winter. On the other hand, statistical analysis revealed that the rate of contamination was significantly higher ($p < 0.001$) in spring and summer than in autumn and winter. Our finding is consistent with previous studies that reported higher rate of parasitic contamination in vegetables during warm seasons than those during cold seasons.[42,53] It has been determined that the excretion of parasite's eggs to environment by human or animals is high in warm seasons comparing to cold seasons.[60] Also, the frequent use of untreated wastewater for irrigation of vegetable fields in Alexandria during spring and summer could be a reason for higher rate of parasitic contamination in these seasons. Another reason might be the difference in weather as oocysts thrive in tropical countries. They are even found to increase in summer season.[29]

5. Conclusion

These findings may have important implications for global food safety and emphasize the importance of raw vegetables in threatening public health by transmission of intestinal parasites to humans in Alexandria.

The local health and environmental authorities should improve the sanitary conditions in the areas where the vegetables are cultivated and consumed. Proper treatment of wastewater used for irrigation of vegetables should be implemented. There is also dire need for the improvement of sanitary facilities in our markets and vegetable vendors. Media programs should inform the consumers the potential health consequences of the intestinal parasites through consumption of raw vegetables, and the importance of proper washing and disinfecting of vegetables before consumption. In addition, they should focus on the necessity of good sanitation hygiene and risks of acquiring intestinal parasites. More researches are needed to do surveys of parasitic contamination in green vegetables in Alexandria including different districts. Also, other surveys must be done in different governorates in Egypt. In these researches, viability of the contaminating parasites or larvation and sporulation of the detected parasites can be done. Also, other researches must be done to evaluate the level of contamination of irrigation water and soil in which green vegetables are cultivated. Different ways of disinfection of raw green vegetables must be improved.

References

1. Van Duyn MA, Pivonka E. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *J Am Diet Assoc* 2000;**100**(12):1511–21.
2. Kniel KE, Lindsay DS, Sumner SS, Hackney CR, Pierson MD, Dubey JP. Examination of attachment and survival of *Toxoplasma gondii* oocysts on raspberries and blueberries. *J Parasitol* 2002;**88**:790–3.
3. Slifko TR, Smith HV, Rose JB. Emerging parasite zoonoses associated with water and food. *Int J Parasitol* 2000;**30**(12–13):1379–93.
4. Mahvi AH, Kia EB. Helminth eggs in raw and treated wastewater in the Islamic Republic of Iran. *East Mediterr Health J* 2006;**12**(1–2):137–43.
5. Beuchat LR. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infect* 2002;**4**(4):413–23.
6. Gupta S, Satpati S, Nayek S, Garai D. Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes. *Environ Monit Assess* 2010;**165**(1–4):169–77.
7. WHO, World Health Organization, Food borne Diseases, Emerging, (2002) <http://www.who.int/mediacentre/factsheets/fs124/en/print.html>.
8. Orlandi PA, Chu DMT, Bier JW, Jackson GJ. Parasites and the food supply. *Food Technol* 2002;**56**:72–81.
9. Dorny P, Praet N, Deckers N, Gabriel S. Emerging food-borne parasites. *Vet Parasitol* 2009;**163**(3):196–206.
10. Jaykus LA. Epidemiology and detection as options for control of viral and parasitic foodborne disease. *Emerg Infect Dis* 1997;**3**:529–39.
11. Gaspard PG, Schwartzbrod J. Parasite contamination (helminth eggs) in sludge treatment plants: definition of a sampling strategy. *Int J Hyg Environ Health* 2003;**206**(2):117–22.

12. Garcia LS. Macroscopic and microscopic examination of fecal specimens. In: Garcia LS, editor. *Diagnostic medical parasitology*. 5th ed. Washington, DC: American Society of Microbiology (ASM); 2007. p. 782–830.
13. Downes FP, Ito K. *Compendium of methods for the microbiological examination of foods*. 4th ed. Washington, DC: American Public Health Association; 2001.
14. Brondson MA. Rapid dimethyl modified acid fast stain of *Cryptosporidium* oocyst in stool specimens. *J Clin Microbiol* 1984;**19**:952–5.
15. Kokoskin E, Gyorkos TW, Camas A, Candiotti L, Purtill T, Ward B. Modified technique for efficient detection of *Microsporidia*. *J Clin Microbiol* 1994;**32**:1074–5.
16. Nyarango RM, Aloo PA, Kabiru EW, Nyanchong BO. The risk of pathogenic intestinal parasite infections in Kisii Municipality, Kenya. *BMC Public Health* 2008;**14**(8):237.
17. Pires SM, Vieira AR, Perez E, Lo Fo, Wong D, Hald T. Attributing human foodborne illness to food sources and water in Latin America and the Caribbean using data from outbreak investigations. *Int J Food Microbiol* 2012;**152**(3):129–38.
18. Mercanoglu Taban B, Halkman AK. Do leafy green vegetables and their ready-to-eat RTE salads carry a risk of foodborne pathogens? *Anaerobe* 2011;**17**(6):286–7.
19. Alexandria- Wikipedia, the free encyclopedia. <http://en.wikipedia.org/wiki/Alexandria>.
20. Amoah P, Drechsel P, Abaidoo RC, Ntow WJ. Pesticide and pathogen contamination of vegetables in Ghana's urban markets. *Arch Environ Con Tox* 2006;**50**(1):1–6.
21. Damen JG, Banwat EB, Egah DZ, Allanana JA. Parasitic contamination of vegetables in Jos. *Nigeria Ann Afr Med* 2007;**6**:115–8.
22. Ettehad GH, Sharif M, Ghorbani L, Ziaei H. Prevalence of intestinal parasites in vegetables consumed in Ardabil. *Iran Food Control* 2008;**19**:790–4.
23. Abougraina AK, Nahaisi MH, Madia NS, Saied MM, Ghengheshc KS. Parasitological contamination in salad vegetables in Tripoli – Libya. *Iran Food Control* 2010;**21**:760–2.
24. Al-Binali AM, Bello CS, El-Shewy K, Abdulla SE. The prevalence of parasites in commonly used leafy vegetables in South Western, Saudi Arabia. *Saudi Med J* 2006;**27**:613–6.
25. Fallah AA, Pirali-Kheirabadi K, Fatemeh S, Saei-Dehkordi S. Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Iran Food Control* 2012;**25**(2):617–20.
26. Shahnazi M, Jafari-Sabet M. Prevalence of parasitic contamination of raw vegetables in villages of Qazvin Province. *Iran Foodborne Pathog Dis* 2010;**7**(9):1025–30.
27. Macarisin D, Bauchan G, Fayer R. Spinacia oleracea L. Leaf stomata harboring *Cryptosporidium parvum* oocysts: a potential threat to food safety. *Appl Environ Microbiol* 2010;**76**:555–9.
28. Stakeholders Analysis Report, City of Alexandria, Egypt By: Center for Environment and Development in the Arab Region and Europe (CEDARE) October 2007. <http://switch.cedare.int/files28%5CFile2830.pdf>.
29. Nabil RA. Detection of Cryptosporidium spp. in different water samples in Alexandria using Real time PCR. MD thesis, Faculty of Medicine, Alexandria University, Egypt, 2012. Unpublished results.
30. Ethelberg S, Lisby M, Vestergaard LS, Enemark HL, Olsen KE, Stensvold CR. A foodborne outbreak of *Cryptosporidium hominis* infection. *Epidemiol Infect* 2009;**137**(3):348–56.
31. Insulander M, de Jong B, Svenungsson B. A food-borne outbreak of cryptosporidiosis among guests and staff at a hotel restaurant in Stockholm county, Sweden, September 2008. Euro Surveill, 2008; 13(51): pii = 19071.
32. Robertson LJ, Gjerde B. Occurrence of parasites on fruits and vegetables in Norway. *J Food Prot* 2001;**64**(11):1793–8.
33. Calvo MM, Carazo ML, Arias C, Chaves R, Monge, Chinchilla M. Prevalence of *Cyclospora* spp, *Cryptosporidium* spp., *Microsporidia* and fecal coliform determination in fresh fruit and vegetables consumed in Costa Rica. *Arch Latinoam Nutr* 2004;**54**:428–32.
34. Amorós I, Alonso JL, Cuesta G. *Cryptosporidium* oocysts and *Giardia* cysts on salad products irrigated with contaminated water. *J Food Prot* 2010;**73**:1138–40.
35. Decraene V, Lebbad Botero-Kleiven MS, Gustavsso AM, Löfdahl M. First reported foodborne outbreak associated with *Microsporidia* Sweden October 2009. *Epidemiol Infect* 2012;**140**(3):519–27.
36. Graczyk TK, Lucy FE, Tamang L, Miraflor A. Human enteropathogen load in activated sewage sludge and corresponding sewage sludge end products. *Appl Environ Microbiol* 2007;**73**:2013–5.
37. Thurston-Enriquez JA, Watt P, Dowd SE, Enriquez R, Pepper IL, Gerba CP. Detection of protozoan parasites and *Microsporidia* in irrigation waters used for crop production. *J Food Prot* 2002;**65**:378–82.
38. Dowd SE, Gerba CP, Pepper IL. Confirmation of the human-pathogenic *Microsporidia Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Vittaforma corneae* in water. *Appl Environ Microbiol* 1998;**64**:3332–5.
39. Fournier S, Liguory O, Santillana-Hayat M, Guillot E, Sarfati C, Dumoutier N, et al. Detection of *Microsporidia* in surface water: a one-year follow-up study. *FEMS Immunol Med Microbiol* 2000;**29**:95–100.
40. Jedrzejewski S, Graczyk TK, Slodkowicz-Kowalska A, Tamang L, Majewska AC. Quantitative assessment of contamination of fresh food produce of various retail types by human-virulent *Microsporidian* spores. *Appl Environ Microb* 2007;**73**(12):4071–3.
41. Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C, et al. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *Am J Trop Med Hyg* 1997;**57**:683–6.
42. Abou el Naga I. Studies on a newly emerging protozoal pathogen: *Cyclospora cayetanensis*. *J. Egypt Soc Parasitol* 1999;**29**:575–86.
43. Sherchand JB, Cross JH, Jimba M, Sherchand S, Shrestha MP. Study of *Cyclospora cayetanensis* in health care facilities, sewage water and green leafy vegetables in Nepal. *Southeast Asian J Trop Med Public Health* 1999;**30**:58–63.
44. Döller PC, Dietrich Karl, Filipp N, Brockmann S, Dreweck C, Vonthein R, et al. Cyclosporiasis outbreak in Germany associated with the consumption of salad. *Emerg Infect Dis* 2002;**8**(9):992–4.
45. Ho AY, Lopez AS, Eberhart MG, Levenson R, Finkel BS, Silva AJ, et al. Outbreak of cyclosporiasis associated with imported raspberries, Philadelphia, Pennsylvania. *Emerg Infect Dis* 2000;**8**(8):783–8.
46. Lopez AS, Dodson DR, Arrowood MJ, Orlandi PA, da Silva AJ, Bier JW, et al. Outbreak of cyclosporiasis associated with basil in Missouri in 1999. *Clin Infect Dis* 2001;**32**:1010–7.
47. Gupta N, Khan DK, Santra SC. Prevalence of intestinal helminth eggs on vegetables grown in wastewater–irrigated areas of Titagarh, West Bengal. *India Food Control* 2009;**20**:942–5.
48. Zohour A, Molazadeh P. Prevalence of pathogenic parasites in consumed vegetables in Jiruft. *J Birj Univ Med Sci* 2001;**8**:10–3.
49. Ulukanligil M, Seyrek A, Aslan G, Ozbilge H, Atay S. Environmental pollution with soil-transmitted helminthes in Sanliurfa. *Turkey Mem I Oswal Cruz* 2001;**96**:903–9.
50. Mogahed NF. A study of medically important soil transmitted parasites in Alexandria, thesis, MS. Faculty of Medicine, University of Alexandria, Egypt, 2010.
51. Kishk NA, Allam SR. Prevalence of intestinal helminthes eggs among preschool children and its relation to soil contamination in Alexandria. *J Med Res Inst* 2000;**21**(1):181–92.
52. Gaspard P, Wiart J, Schwartzbrod J. Parasitological contamination of urban sludge used for agricultural purposes. *Wast Manage Res* 1997;**15**:429–36.

53. Kozan E, Sevimli FK, Köse M, Eser M, Ciçek H. Examination of helminth contaminated wastewaters used for agricultural purposes in Afyonkarahisar. *Turkiye Parazitol Derg* 2007;**31**(3):197–200.
54. Avcioğlu H, Soykan E, Tarakci U. Control of helminth contamination of raw vegetables by washing. *Vector Borne Zoonotic Dis* 2011;**11**(2):189–91.
55. Taherian F. Prevalence of Giardia lamblia in healthy people in Shahrekord, Institute of Public Health, Shahrekord University of Medical Sciences Fact Sheet FA-93.2009.
56. Gharavi MJ, Jahani MR, Rokni MB. Parasitic contamination of vegetables from farms and markets in Tehran. *Iran J Pub Health* 2002;**31**:83–6.
57. Shahnazi M, Sharifi M, Kalantari Z, Heidari MA, Agamirkarimi N. The study of consumed vegetable parasitic infections in Qazvin. *JQUMS* 2009;**12**(4):83.
58. Al-Megrin AI. Prevalence of intestinal parasites in leafy vegetables in Riyadh. *Saudi Arabia Int J Trop Med* 2010;**5**:20–3.
59. Erdoğru Ö, Şener H. The contamination of various fruit and vegetable with *Enterobius vermicularis*, *Ascaris eggs*, *Entamoeba histolyca cysts* and *Giardia cysts*. *Iran Food Control* 2005;**16**:559–62.
60. Eslami A, Rahbari S, Ranjbar-Bahadori S, Kamal A. Study on the Prevalence Seasonal incidence and economic importance of Parasitic infections of small ruminants in the province of Semnan. *Pajouhesh Sazandegi* 2003;**58**:55–8.