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Prevalence of enteric Protozoa on fresh vegetables and their irrigation water in Giza, Egypt

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ABSTRACT

Cryptosporidium spp., *Entamoeba histolytica*, and microsporidia are the most common enteric protozoans acting as parasitic agents of waterborne diseases. They are considered to be responsible for human infections. Fresh vegetables are one of the most important vehicles of foodborne protozoan parasitic disease transmission. Vegetables are contaminated with enteric Protozoa from the beginning of the planting process to the consumption. In the present study, a total of 110 vegetable samples were collected from agriculture fields (3 regions; two from Nahia and one from Saft Al-Laban, Giza governorate, Egypt). Moreover, 36 irrigation water samples (ground and surface freshwater) were collected from the same agriculture fields. Another group of 109 vegetable samples was collected from the public markets in Dokki district, Giza governorate, Egypt. Each sample was separately processed and examined for determining the prevalence of enteric Protozoa. The results showed that 39 (35.5%) out of 110 field-collected vegetable samples from three agriculture field areas, 37 (33.9%) out of 109 vegetable samples from markets, and 7 (19.4%) out of 36 irrigation water samples were positive for the three intestinal Protozoa. The most contaminated vegetables were those collected from Nahia 1 area (irrigated with surface water) 41.9%, followed by the Saft area (irrigated with groundwater) 34.8% and Nahia 2 area (irrigated with groundwater) (32.1%). The most contaminated irrigation water was the surface water collected from Nahia 1 area (50%) followed by ground irrigation water in Saft Al-Laban (8.3%). No contamination in groundwater of Nahia 2 area. The most contaminated vegetable was dill collected from both filed and market samples (71.4% and 66.7%, respectively). The most dominant type of enteric parasite was microsporidia spores, on-field vegetables (18.2%), market vegetables (18.3%), irrigation water (11.1%); the last was also contaminated with *Cryptosporidium* oocysts (11.1%).

INTRODUCTION

Enteric protozoans are unicellular parasites inhabiting the gastrointestinal tract of many vertebrates. Globally, 60% of people worldwide were infected with intestinal parasites (**Adamu *et al*., 2013**). Intestinal protozoan infections were documented among both immunosuppressed and immunocompetent patients (**Fletcher *et al*., 2012; Han and Weiss, 2017**). *Cryptosporidium*, *Entamoeba* and microsporidia are considered as major intestinal Protozoa of public health importance (**Kosek *et al*., 2001 ; Haque *et al*., 2003**; **Han and Weiss, 2017; Daniel *et al*., 2018; Kimosop *et al*., 2019**; **Ögren *et al*., 2020**) due to being responsible for waterborne diseases in human.

The consumption of freshly-eaten vegetables without proper washing is an important route in the transmission of parasitic diseases (**Slifko *et al*., 2000**). Transmission of *Cryptosporidium* occurs easily through person-to-person and immediately as the oocysts are excreted fully sporulated in stool (**Elwin *et al*., 2012**).

In Egypt, the most common infectious of protozoans, which cause gastrointestinal troubles are *Entamoeba histolytica* (**El-Kadi *et al*., 2006; Bayoumy *et al.*, 2010**; **Nazeer *et al*., 2013)** and *Cryptosporidium* spp. (**Abdel-Hafeez *et al.*, 2012; Saleh *et al*., 2019**). The main objectives of this study were to survey enteric protozoan parasites harbored in both vegetables' samples (fields/markets) and irrigation water (ground/surface water) from 3 different areas (two in Nahia and one in Saft El-Laban). Besides, comparing the prevalence of 3 enteric protozoan parasites (*Cryptosporidium*, *Entamoeba* sp. and microsporidia) in the field and market-collected vegetable samples and consequently, in the above listed irrigation systems. Study the seasonal variations in the prevalence of the three enteric protozoans for 2 successive years was tracked.

MATERIALS AND METHODS

1. *Locations of collected samples*

The present study was carried out in Nahia and Saft Al-Laban areas (Giza governorate, Egypt). Nahia area has agriculture fields irrigated either with surface freshwater from Maryotia canal (Nahia 1) or with groundwater (Nahia 2). Saft Al-Laban area has agriculture fields irrigated only with groundwater. Some common freshly-eaten vegetables were collected from public markets in Dokki district, Giza, Egypt.

2. *Collection of samples*

Freshly eaten vegetables were collected from Nahia1, 2, Saft Al-Laban and public markets in Dokki district. The collected vegetable samples were dill (*Anethum graveolens*), parsley (*Petroselinum crispum*), watercress (*Nasturtium officinale*), tomatoes (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*), white radish (*Raphanus sativus var*. *longipinnatus*), green onion (*Allium cepa*), and cucumber (*Cucumis sativus*). Fresh vegetable samples (500g each) were separately collected in clean transparent nylon bags.

Irrigation water samples were collected from Nahia and Saft Al-Laban regions, using 20L sterile polypropylene containers. All samples were transferred on the same day of collection to Environmental Parasitology Laboratory, National Research Centre, Dokki, Giza, Egypt.

3. *Collection of Parasites*

3.1. Irrigation-water samples:

The water sample was filtered through a stainless steel pressure filter holder (Sartorius) using nitrocellulose membrane filter (0.45µm pore size and 142mm diameter) **(Brandonisio *et al.*, 2000).** The membrane filter was washed 3 times (each with sterile physiological saline). The washing solution was centrifuged at 2000 rpm for 5 min **(WHO, 2000; Kwakye-nuako *et al*., 2007).** The supernatant was discarded, the debris, containing different parasitic stages was separately subjected to flotation method according to **Dryden *et al*., 2005.** The top reverse meniscus (in Eppendorf tube) was prepared for identifying parasites by the microscopic examination.

3.2. ***Fresh vegetables:***

The vegetable sample was transmitted to a large clean plastic container, then washed twice; firstly, with 2L distilled water and secondly with 2L detergent solution **(Luz *et al*., 2017)** with vigorous shaking for 15min (**Al-Shawa and Mwafy, 2007)**. After removal of the washed vegetable sample, the remaining water of washing was managed the same as previously mentioned for irrigation water samples.

4. Microscopic examination

Eppendorf tube content was centrifuged at ٢٠٠٠rpm for ٥min .The supernatant was discarded and remaining was dropped on two separate clean glass slides. The first slide was treated with a 20µL of Lugol's Iodine solution and examined with 40X objective lens of the research microscope (**Garcia and Bruckner, 1997**). The second slide was left for air-drying in room temperature, fixed with methanol 95% for 15min and stained with acid-fast trichrome (AFT) stain according to **Ignatius *et al.* (1997)**. The stained smears were microscopically examined using 100X oil-lens magnification.

5. Statistical analysis.

The obtained data were statistically analyzed using Minitab Statistical Program (**Meyer and Krueger, 2004**).

RESULTS

Microscopically, 3 different enteric protozoan parasites were detected (*E. histolytica* cysts, microsporidial spores and *Cryptosporidium* oocysts) on vegetable-washing water and in vegetable-irrigation water.

Out of 110 and 109 vegetable samples collected from agriculture fields and markets respectively, 39 (35.5%) and 37 (33.9%) were positive for intestinal Protozoa, respectively. Seven samples (19.4%) out of 36 irrigation water samples from the same agriculture fields were positive for intestinal Protozoa (Table 1 & Fig.1a).

Examination of vegetables collected from the agriculture fields revealed that Nahia1 area (irrigated with surface water) was the most contaminated area with intestinal protozoon parasites (41.9%), followed by Saft Al-Laban area (irrigated with groundwater) 34.8% and Nahia2 area (irrigated with groundwater) (32.1%) (Table1 & Fig.1 b). About 50% of surface irrigation water samples in Nahia1 area were found to be contaminated with intestinal Protozoa, followed by 8.3% of ground irrigation water of Saft Al-Laban area and no contamination in groundwater of Nahia2 area (Table1 & Fig.1 b). Statistically, there was a significant difference in prevalence of total detected protozoan parasites between the surface and ground irrigation water (where P-value = 0.010).

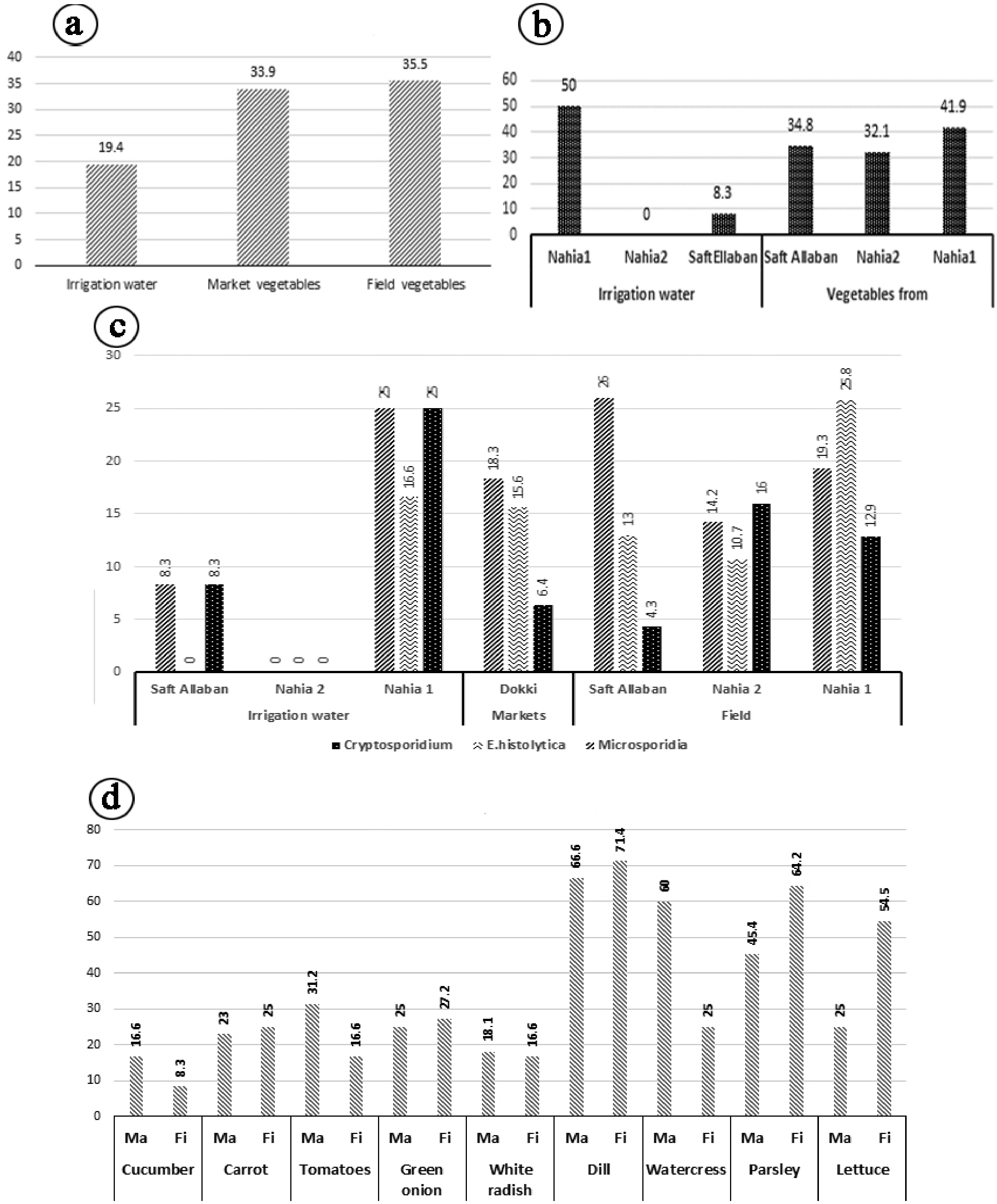

Fig.1 a) Prevalence of enteric protozoan parasites on vegetable and irrigation water samples. b) Prevalence of enteric Protozoa in vegetables and irrigation water from different field areas. c) Prevalence of enteric protozoan parasites in vegetables and irrigation water from different field areas. d) Prevalence of enteric Protozoa on different types of examined vegetables from field (Fi) and markets (Ma).

Concerning the parasites, microsporidia has the highest prevalence rates of enteric Protozoa on field and market vegetables (18.2% & 18.3%), followed by *Entamoeba* (15.5% and 15.6%) and *Cryptosporidium* (12.7% and 6.4%), respectively, while in irrigation water, the prevalence rates were the same in both microsporidia and *Cryptosporidium* (11.1%), followed by *Entamoeba* (5.6%) (Table1). Statistically, there was no significant difference of *Entamoeba histolytica* prevalence between field and market vegetables (where P-value = 0.631). In addition, there was no significant difference of *Cryptosporidium* prevalence between field and market vegetables (where P-value = 0.138). Concerning microsporidia, there was a significant difference of their prevalence between field and market vegetables (where P-value = 0.803).

Concerning the highest prevalence rate for each parasite in different field areas, microsporidia spores in Saft Al-Laban area (26%), *E. histolytica* cysts in Nahia 1 (25.8%) and *Cryptosporidium* oocysts in Nahia 2 area (16%). However, the lowest prevalence rates with parasites in different field areas, *Cryptosporidium* oocysts in Saft Al-Laban area (4.3%), *E. histolytica* cysts (10.7%) and *Cryptosporidium* in Nahia1 area (12.9%). (Fig.1 c)

Concerning irrigation water, *Cryptosporidium* and microsporidia had the same prevalence rates in ground irrigation water of Saft area (8.3%) and in water surface of Nahia 1 (25%). No protozoan infections were detected in groundwater of Nahia 2 (Fig.1 c). Statistically, there was a significant difference in prevalence of total detected protozoan parasites between the surface and ground irrigation water (where P-value = 0.010). The most contaminated vegetable with intestinal Protozoa was dill in both field and market samples (71.4% and 66.7%, respectively). The lowest contaminated vegetable was found to be cucumber in both field and market (8.3% and 16.6%, respectively) (Table 2 & Fig.1 d).

The pairs of field vegetables (watercress/carrot and white-radish/tomatoes) were contaminated with similar percent of intestinal Protozoa (25% and 16.7%, respectively), likewise, the market vegetables Lettuce/ Green-onion were (25%) (Table 2 & Fig.1d).

In field vegetables, the highest prevalence rates by *E. histolytica* was in parsley (35.7%), *Cryptosporidium* in dill (42.8%) and by microsporidia in lettuce (45.4%). No contamination with *E. histolytica* cysts on both green onion and tomatoes; *Cryptosporidium* oocysts on lettuce, green onion, tomatoes and cucumber and no microsporidia spores on cucumber. (Table 2 & Fig.2 a).

The prevalence rates were equal by *E. histolytica* cysts in watercress, white radish and cucumber (8.3%) *Cryptosporidium* in watercress and white radish (16.7%) and microsporidia in watercress and white radish (8.3). (Table 2 & Fig.2 a)

In market vegetables, the highest prevalence rates by *E. histolytica* was in dill (33.3%), *Cryptosporidium* in carrot (15.4%) and by microsporidia in dill (33.3%). No contamination with *E. histolytica* cysts on carrot; *Cryptosporidium* oocysts on watercress, dill, green onion and cucumber and no microsporidia spores on cucumber. (Table 2 & Fig.2 a)

Concerning seasonal variation, the highest prevalence rates of enteric Protozoa in field vegetables were recorded in winter (44.8%) (Table 3)**,** while in market vegetables, the highest prevalence rates were in spring (42.8%) (Table 4 & Fig. 2b)**.** In irrigation water, the highest prevalence rates of present Protozoa were 22.2% in spring, winter and summer. (Table 5)

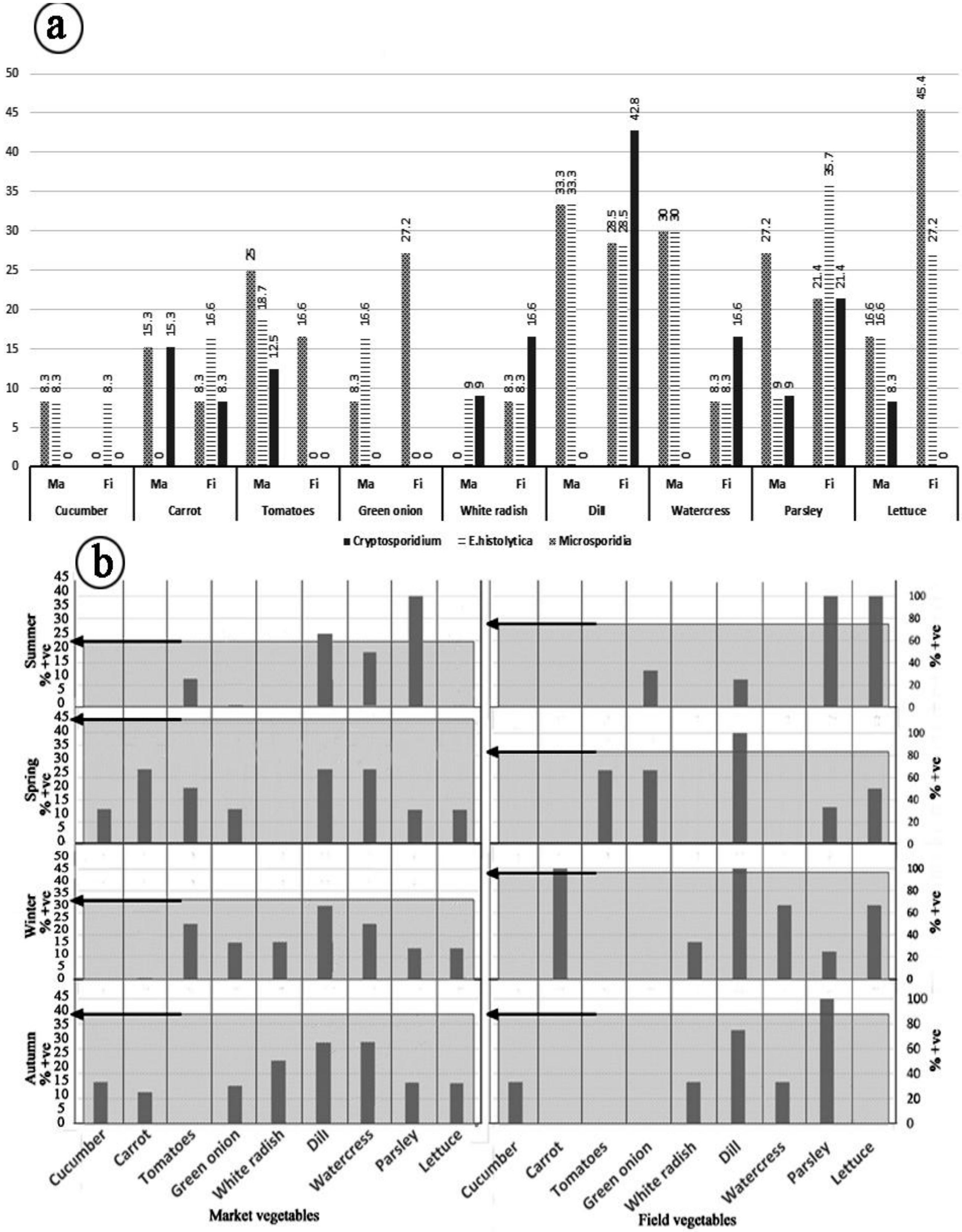

Fig. 2. a) Prevalence of different enteric protozoan parasites on vegetables from field (Fi) and markets (Ma). b) Seasonal prevalence of enteric protozoan parasites on different examined field/market vegetable samples (narrow bars) and seasonal variation in general prevalence of both field and market vegetable samples (broad bars).

Table 1. Prevalence of different enteric protozoan parasites on examined vegetables and irrigation water samples from different field areas.

	Vegetables						Irrigation water			
	Field				Markets		Water surface	Ground water		
	Nahia 1	Nahia 2	Saft Al-Laban		Dokki		Nahia 1	Nahia 2	Saft Al-Laban	
Parasite	**+ ve (%)**	**+ ve (%)**	**+ ve (%)**	**Total +ve Per parasite / %**	**+ve**	**Total +ve Per parasite / %**	**ve + (%)**	**ve + (%)**	**ve + (%)**	**Total +ve Per parasite / %**
Cryptosporidium	4(12.9)	9(16)	1(4.3)	14/12.7	7	7/6.4	3(25)	0(0)	1(8.3)	4/11.1
E. histolytica	8(25.8)	6(10.7)	3(13)	17/15.5	17	17/15.6	2(16.6)	0(0)	0(0)	2/5.6
Microsporidia	6(19.3)	8(14.2)	6(26)	20/18.2	20	20/18.3	3(25)	0(0)	1(8.3)	4/11.1
No. samples/area (+ve/%)	31 (13/41.9)	56 (18/32.1)	23 (8/34.8)		109 (37)		12[a] (6/50)	12[b] (0/0)	12[b] (1/8.3)	
Total sample (+ve/%)	110* (39/35.5%)				109** (37/33.9%)		36 (7/19.4%)			

* For *Entamoeba*: Mean=0.83, S.D.= 2.25, SE Mean=0.21
For *Cryptosporidium*: Mean=0.79, S.D.= 2.56, SE Mean=0.
For microsporidia: Mean=1.83, S.D.= 4.71, SE Mean=0.45
** For *Entamoeba*: Mean=0.69, S.D.= 2.03, SE Mean=0.19
For *Cryptosporidium*: Mean=0.37, S.D.= 1.52, SE Mean=0.15
For microsporidia: Mean=2.00, S.D.= 5.50, SE Mean=0.53
a: Mean=4.67, S.D.= 6.68, SE Mean=1.9
b: Mean=0.50, S.D.= 2.45, SE Mean=0.50

Table 2. Prevalence of different enteric protozoan parasites in different types of field (FI*) and market (Ma*) vegetables.

Parasite	Lettuce +ve/%		Parsley +ve/%		Watercress +ve/%		Dill +ve/%		White radish +ve/%		Green onion +ve/%		Tomatoes +ve/%		Carrot +ve/%		Cucumber +ve/%	
	Fi*	Ma*	Fi*	Ma*	Fi*	Ma*	Fi*	Ma*	Fi*	Ma*	Fi*	Ma*	Fi*	Ma*	Fi*	Ma*	Fi*	Ma*
Cryptosporidium	0/0	1/8.3	3/21.4	1/9	2/16.6	0/0	6/42.8	0/0	2/16.6	1/9	0/0	0/0	0/0	2/12.5	1/8.3	2/15.3	0/0	0/0
E. histolytica	3/27.2	2/16.6	5/35.7	1/9	1/8.3	3/30	4/28.5	4/33.3	1/8.3	1/9	0/0	2/16.6	0/0	3/18.7	2/16.6	0/0	1/8.3	1/8.3
Microsporidia	5/45.4	2/16.6	3/21.4	3/27.2	1/8.3	3/30	4/28.5	4/33.3	1/8.3	0/0	3/27.2	1/8.3	2/16.6	4/25	1/8.3	2/15.3	0/0	1/8.3
Total exam.	11	12	14	11	12	10	14	12	12	11	11	12	12	16	12	13	12	12
Total +ve (%)	6 (54.5)	3 (25)	9 (64.2)	5 (45.4)	3 (25)	6 (60)	10 (71.4)	8 (66.6)	2 (16.6)	2 (18.1)	3 (27.2)	3 (25)	2 (16.6)	5 (31.2)	3 (25)	3 (23)	1 (8.3)	2 (16.6)

Table 3. Seasonal prevalence of different enteric protozoan parasites on examined field vegetable samples.

Season		Field samples										+ve each parasite (%)	Parasite
		Lettuce	Parsley	Water cress	Dill	White radish	Green onion	Tomatoes	Carrot	Cucumber	Total		
Autumn	**No. of Exam. /type**	2	4	3	4	3	2	3	3	3	27		
	+ve (%)	0 (0)	4 (100)	1 (33.3)	3 (75)	1 (33.3)	0 (0)	0 (0)	0 (0)	1 (33.3)	10 37	6 (22.2)	***Cryptosporidium***
												4 (14.8)	***E. histolytica***
												3 (11.1)	**Microsporidia**
Winter	**No. of Exam. /type**	3	4	3	4	3	3	3	3	3	29		
	+ve (%)	2 (66.6)	1 (25)	2 (66.6)	4 (100)	1 (33.3)	0 (0)	0 (0)	3 (100)	0 (0)	13 44.8	6 (20.6)	***Cryptosporidium***
												9 (31)	***E. histolytica***
												5 (17.2)	**Microsporidia**
Spring	**No. of Exam. /type**	4	3	3	2	5	3	3	3	3	29		
	+ve (%)	2 (50)	1 (33.3)	0 (0)	2 (100)	0 (0)	2 (66.6)	2 (66.6)	0 (0)	0 (0)	9 31	2 (6.8)	***Cryptosporidium***
												2 (6.8)	***E. histolytica***
												6 (20.6)	**Microsporidia**
Summer	**No. of Exam. /type**	2	3	3	4	1	3	3	3	3	25		
	+ve (%)	2 (100)	3 (100)	0 (0)	1 (25)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	7 28	0 (0)	***Cryptosporidium***
												2 (8)	***E. histolytica***
												6 (24)	**Microsporidia**

Table 4. Seasonal prevalence of different enteric protozoan parasites on examined market vegetable samples.

Season		Market samples: Lettuce	Parsley	Watercress	Dill	White radish	Green onion	Tomatoes	Carrot	Cucumber	Total	+ve /parasite (%)	Parasite
Autumn	No. of Exam./type	3	3	3	3	2	3	4	4	3	27		
	+ve (%)	1 (33.3)	1 (33.3)	2 (66.6)	2 (66.6)	1 (50)	1 (33.3)	0 (0)	1 (25)	1 (33.3)	10 37	2 (7.4)	***Cryptosporidium***
												5 (18.5)	***E. histolytica***
												3 (11.1)	**Microsporidia**
Winter	No. of Exam./type	3	3	2	3	3	3	4	3	3	27		
	+ve (%)	1 (33.3)	1 (33.3)	1 (50)	2 (66.6)	1 (33.3)	1 (33.3)	2 (50)	0 (0)	0 (0)	9 33.3	3 (11.1)	***Cryptosporidium***
												5 (18.5)	***E. histolytica***
												4 (14.8)	**Microsporidia**
Spring	No. of Exam. /type	3	3	3	3	3	3	4	3	3	28		
	+ve (%)	1 (33.3)	1 (33.3)	2 (66.6)	2 (66.6)	0 (0)	1 (33.3)	2 (50)	2 (66.6)	1 (33.3)	12 42.8	2 (7.1)	***Cryptosporidium***
												4 (14.2)	***E. histolytica***
												8 (28.5)	**Microsporidia**
Summer	No. of Exam. /type	3	2	2	3	3	3	4	3	3	27		
	+ve (%)	0 (0)	2 (100)	1 (50)	2 (66.6)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	6 22.2	0 (0)	***Cryptosporidium***
												2 (7.4)	***E. histolytica***
												5 (18.5)	**Microsporidia**

Table 5. Seasonal prevalence of different enteric protozoan parasites on examined irrigation water samples.

Season	water samples		+ve/parasite(%)	Parasite
Autumn	Exam. No.	9		
	+ve (%)	1 (11.1)	1 (11.1)	*Cryptosporidium*
			0 (0)	*E. histolytica*
			0 (0)	Microsporidia
Winter	Exam. No.	9		
	+ve (%)	2 (22.2)	1(11.1)	*Cryptosporidium*
			1 (11.1)	*E. histolytica*
			1 (11.1)	Microsporidia
Spring	Exam. No.	9		
	+ve (%)	2 (22.2)	1 (11.1)	*Cryptosporidium*
			1 (11.1)	*E. histolytica*
			1 (11.1)	Microsporidia
Summer	Exam. No.	9		
	+ve (%)	2 (22.2)	1 (11.1)	*Cryptosporidium*
			0 (0)	*E. histolytica*
			2 (22.2)	Microsporidia

Concerning seasonal variation in prevalence rates assigned to each parasite, in field vegetables, the highest prevalence of *E. histolytica* cysts reached 31% in winter; *Cryptosporidium* oocysts, 22.2 % in autumn and microsporidia spores, 24% in summer (Table 3). On the other hand, in market vegetables, the highest prevalence of *E. histolytica* cysts reached (18.5%) in both autumn and winter, *Cryptosporidium* oocysts prevailed in winter (11.1%) and microsporidia spores with the highest prevalence 28.6% in spring. (Table 4). In irrigation water samples, the highest prevalence rate of *E. histolytica* cysts reached 11.1% in both winter and spring but no contamination was detected in both autumn and summer. The prevalence rate of *Cryptosporidium* oocysts was constant (11.1%) all over the year, while the highest prevalence rate of microsporidia spores reached 22.2% in summer with no contamination recorded in autumn to the same parasite.

According to seasonal variations in the prevalence of different field vegetables, in autumn, the highest prevalence of present parasites was on parsley (100%), in winter, was found to be 100% on both dill and carrot, in spring, the highest prevalence rate was 100% on dill, in summer, and was also 100% on both lettuce and parsley (Table 4 & Fig. 2b). No contaminations were detected in lettuce, green onion, tomatoes and carrot in summer; green onion, tomatoes and cucumber in winter; watercress, white radish, carrot and cucumber in spring and watercress, white radish, tomatoes, carrot and cucumber in summer. (Table 3& Fig. 2b)

Concerning to seasonal variations in the prevalence of different market vegetables, in autumn, the highest prevalence rate of present parasites was on both watercress and dill (66.6%), in winter, was found to be 66.6% on dill, in spring, the highest prevalence rate was 66.6% on each of watercress, dill and carrot, in summer, it was 100% on parsley. No contaminations were detected in tomatoes in autumn; carrot and cucumber in winter; white radish in spring; lettuce, white radish, green onion, carrot and cucumber in summer. (Table 4 & Fig. 2b)

DISCUSSION

High incidences of intestinal parasites have been found in communities consuming raw vegetables, cultivated on farms fertilized with untreated human and animal fertilizers (**Srikanth and Naik, 2004**). There are many documents published on the contamination of raw vegetables with parasites (**Adamu *et al.*, 2012, Medeiros *et al.*, 2019**).

In the present study, the prevalence of *Entamoeba* cysts, *Cryptosporidium* spp. oocysts and spores of intestinal microsporidia in market vegetables reached 15.6%, 6.4% and 18.3%, respectively. A lower prevalence with *Entamoeba* cysts and *Cryptosporidium* oocysts was reported in Ethiopia (14.44 and 4.7%, respectively) (**Bekele *et al*., 2017)**. In Saudi Arabia, *Cryptosporidium* oocysts were encountered on 6.3% of some leafy vegetables **(Al-Binali *et al*., 2006)**. *Entamoeba* cysts were detected on 10.7% of some fresh vegetables collected from small retailers and peddlers in Saudi Arabia **(Ammar and Omar, 2012**) and on 8.75 % of some raw vegetable samples collected from different markets in Syria (**Alhabbal, 2015).** Other studies in Nigeria, **(Idahosa, 2011),** Egypt **(Saleh *et al*., 2018**), Iraq **(Ali and Ameen, 2013),** Iran (**Ebrahimzadeh *et al*., 2013)**, Nigeria (**Ishaku *et al*., 2013**)**,** Ethiopia **(Tefera *et al*., 2014)** and Lahore (**Shafa-ul-Haq *et al.***, ٢٠١٤) detected *Entamoeba* spp. on 6.8، 6.4, 14.66, 15, 7.1, 5.3 and ٧.٠٥% of examined fresh vegetables, respectively. It was suggested that differences in prevalence rates between investigations are expected, probably due the origin of the vegetables and the consequential differences in cultivation, transport and storage of them **(Abougrain *et al*., 2010).**

A higher prevalence of *Cryptosporidium* on some fresh vegetables was reported in Saudi Arabia (13%) **(Ammar and Omar, 2012**), Egypt **(**29.3%) **(Said, 2012)**, Vietnam **(**47.22%) **(Chau *et al*., 2014)** and Ethiopia (12.8%) **(Tefera *et al*., 2014)**. In addition, a higher prevalence of microsporidia on some fresh vegetables was reported in Egypt **(Said, 2012)** in a percentage reaching ٢٥.٣ %.

In the present study, the highest prevalence of *Entamoeba* cysts on field vegetables was found in parsley (35.7%), followed by dill, lettuce and carrot (28.6, 27.3 and 16.7%, respectively). About 8.3% of examined watercress, white radish and cucumber were contaminated with *Entamoeba* cysts, but no contamination with *Entamoeba* appeared on both green onion and tomatoes. Other workers in Ethiopia recorded a lower prevalence of *Entamoeba* on lettuce (12.5%), spinach and cabbage (8.3% for each) collected from farms irrigated with wastewater **(Benti and Gemechu, 2014).**

The contamination of vegetables with enteric Protozoa may occur by contacting with the soil, raw manure and sewage used as fertilizer for vegetable farms. In most cases, contamination of vegetables was associated with the water used for irrigation **(Simoes *et al*., 2001)**. Use of water contaminated with sewage for irrigation of vegetables is a common practice in developing countries. The access to clean water for irrigation of vegetables is a major challenge. As an alternative, urban and peri-urban vegetable farmers in search of water for their crops have no other choice than to use water from these highly polluted sources **(Benti and Gemechu, 2014)**.

In the present study, the highest prevalence of *Entamoeba* cysts in market vegetables was found on 33.3% of dill, followed by watercress and tomatoes (30 and 18.8%, respectively), lettuce and green onion (16.7% for each), on both parsley and white radish (9.1% for each) and cucumber (8.3%), but no contamination with *Entamoeba* cysts was recorded on carrot. Other workers in Iran reported a lower prevalence of *Entamoeba* cysts on 7% of tomatoes collected from markets **(Yagoob and Mohammad, 2015**).

In the current study, dill is among the most contaminated vegetables in both filed and market samples (71.4% and 66.6%, respectively), this finding was supported by another Egyptian study by Saleh *et al.*, 2018 (21.4 % and 16.7%, respectively) The main factor of contamination of vegetables with different parasitic stages may be due to the rough surface and leaf folds of this vegetable (ex. dill) that can retain dirt and dusts which are not easily washed off (**Islam *et al.,* 2004**). Other types of vegetables may have large surface areas and compact structures (ex. lettuce) that provide a better fixation and facilitate the permanence of parasitic stages on the plant **(Adamu *et al*., 2012)**. Washing of vegetables with clean water before selling, may play an important role in reduction of transmission of parasitic infection to human through consuming such vegetables **(Saki *et al*., 2013)**.

In the present study, the highest prevalence of *Cryptosporidium* in field vegetables was found on dill (42.8%), followed by parsley (21.4%), watercress and white radish (16.6% for each) and carrot (8.3%). No contamination with *Cryptosporidium* was detected on lettuce, green onion, tomatoes and cucumber. Another study in Iran showed a higher prevalence of contamination with *Cryptosporidium* on green onion (14.8 %), cress, mint, coriander, leek and basil (8.9, 8.5, 6.7, 3.3 and 1.1%, respectively) collected from farms of agricultural regions (**Ranjbar-Bahadori *et al.,* 2013**).

In the present study, the highest prevalence of *Cryptosporidium* oocysts in market vegetables was encountered on 15.3% of carrot, followed by tomatoes (12.5%), parsley and white radish (9% for each) and lettuce (8.3%), but no contamination with *Cryptosporidium* was encountered on watercress, dill, green onion and cucumber. Other studies in Vietnam showed a higher prevalence of *Cryptosporidium* on lettuce and watercress (55.6 and 44.4%, respectively)**,** as well as young mustard greens, celery, amaranth, water spinach, cilantro, rice paddy herb, Vietnamese cilantro, basil, centella, and iceberg lettuce (44.4, 33.3, 22.2, 55.6, 33.3, 55.6, 44.4, 55.6, 66.7, and 55.6%, respectively) **(Chau *et al*., 2014)**.

In the present study, the highest prevalence of microsporidia contaminating field vegetables was found on lettuce (45.4%), followed by dill, green onion, parsley and tomatoes (28.5%, 27.2%, 21.4% and 16.6%, respectively), white radish, watercress and carrot (8.3% for each), but no contamination with spores of microsporidia was observed on cucumber. To the best of our knowledge, there were no published data concerning contamination of field vegetables with microsporidia spores until now.

In the present study, the highest contamination with microsporidia spores on market vegetables was found on dill (33.3%), followed by watercress, parsley, tomatoes, lettuce, carrot (30, 27.3, 25, 16.7 and 15.4%, respectively), green onion and cucumber (8.3% for each). No contamination with microsporidia was encountered on white radish. Another study on market vegetables in Egypt showed higher contamination with microsporidia on lettuce, rocket and green onion (41.7, 36.7 and 18.3%, respectively), but a lower prevalence was found on parsley and leek (16.7 and 13.3%, respectively) **(Said, 2012)**. It was found that transport; handling and exhibition at the point of sale could also influence the parasitological contamination of vegetables **(Takayanagui *et al*., 2006).**

Concerning the present enteric protozoan parasites in ground irrigation water, microscopic detection revealed the presence of *Cryptosporidium* and microsporidia had the same prevalence rate (4.1%), while *Entamoeba* cysts were not detected at all. Other studies in Iran showed a higher prevalence of contamination with *Cryptosporidium* in 6% of well irrigation water **(Ranjbar-Bahadori *et al.*, 2013**). With a similar manner, in surface irrigation water, the prevalence rate of microsporidia spores and *Cryptosporidium* oocysts were the same (25%), followed by *Entamoeba* cysts (16.6%). Other study showed a lower prevalence of contamination with *Cryptosporidium* (18.4%) in irrigation water in Spain **(Gracenea *et al*., 2011)**, where the sampling sites of irrigation channels were protected by sidewalls. In Turkey, **Turgay and Sener, 2005** detected *Entamoeba* in canal irrigation water. Studies on surface waters used for irrigation in the United States, Mexico and South Africa showed that (28, 48 and 43%), respectively of the samples analyzed were positive for *Cryptosporidium* (**Thurston-Enriquez *et al*., 2002; Chaidez *et al*., 2005; Duhain, 2011**).

Concerning seasonal variations in the prevalences of enteric Protozoa, in field-vegetables, the highest prevalence rate was found to be 44.8% in winter, followed by autumn, spring and summer (37, 31 and 28%, respectively)**,** while in market vegetables, the highest one was found to be 42.8% in spring, followed by autumn, winter and summer (37%, 33.3% and 22.2%, respectively**).** In comparison with another study in Egypt, **(Said, 2012),** the highest rate of parasitic contamination in vegetable samples collected from markets was found in spring (49.3%), followed by summer, autumn and winter (48, 20 and 9.3%, respectively).

In market vegetables, *Entamoeba* cysts prevailed in both autumn and winter (18.5% for each), followed by spring and summer (14.3 and 7.4%, respectively). This accepted with similar studies in Brasil, indicated that parasites were significantly more frequent in vegetables in the rainy season **(Simoes *et al*., 2001)**. In Iraq, *Entamoeba* spp. were detected in winter vegetables with a prevalence higher than in summer vegetables. In Malaysia, *Entamoeba* spp. were detected in the dry season (February-April) of some market vegetables, but no contamination was found in monsoon season (November-January) **(Yusof *et al*., 2017)**. In Egyptian studies, *Entamoeba* spp. were detected on vegetable samples indicating that the rate of parasitic contamination with *Entamoeba histolytica* was the highest in summer (49%) and the lowest in winter (10.8%) **(Eraky *et al*., 2014**) in another study, (**Saleh *et al.*, 2018**) the highest prevalence was in autumn (7.4%) and no contamination detected in winter's vegetables.

In this study, contamination of irrigation water by *Cryptosporidium* sp. had constant prevalence all over the year. **Shrestha *et al.* (2016)** stated that ground and irrigation water (Nepal) were free from contamination by *Cryptosporidium* in the mid-rainy (August) season, although that parasite's prevalence reaches to 82% in the river water in.

CONCLUSION

Freshly eaten vegetables irrigated with surface water were more contaminated with enteric Protozoa than that irrigated with groundwater. Dill was the most contaminated crop with intestinal Protozoa. The most prevalent protozoan was microsporidia spores, followed by *Entamoeba* cysts and lastly *Cryptosporidium* oocysts. The presence of such protozoan parasites on freshly eaten vegetables represents public health hazards to consumers. The present study emphasizes the need to develop local control measures and preventive interventions guided by the incidence of a particular disease rather than socioeconomic indicators alone. Besides, the expansion in using groundwater in agriculture, especially with increasing of the global water crisis.

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