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Contamination assessment of *Giardia* spp. and *Entamoeba* spp. cysts in fresh green vegetables collected from farms and markets of Koya city, Erbil Province of Kurdistan region, Iraq

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ABSTRACT

Fresh vegetables are the important part of a healthy diet and they are usually contaminated with protozoan parasites. These protozoa can be a threat to the public health by consumption of raw or minimal processing vegetables and they may serve as vehicles for transmission of intestinal protozoan parasites. However, comparative evidence on the occurrence of *Giardia* spp. and *Entamoeba* spp. in vegetables from different points of the food supply chain in Koya City in Iraq remains limited. This study aimed to determine and compare the prevalence and contamination levels of cysts for *Giardia* spp. and *Entamoeba* spp. in fresh vegetables collected directly from farms and retail markets of Koya City, and to assess whether contamination between these two collection places. A cross-sectional study was conducted using fresh vegetables samples collected from selected farms and markets. Four hundred vegetable samples including parsley, leek, radish, celery and green onion were investigated. Samples were processed using appropriate washing, concentration, and microscopic examination procedures for detection of *Giardia* spp. and *Entamoeba* spp. cysts. The prevalence of each parasite was calculated and compared between farm and market collected vegetables. Associations between contamination and collected place and vegetable type was also evaluated using appropriate statistical methods. Results showed that the contamination rate of cysts for *Entamoeba* spp. and *Giardia* spp. were 15% and 10.25%, respectively. Parsley had the highest contamination among the vegetables and it was 4% from *Entamoeba* spp. Also, celery had the highest contamination with 3.75% for *Giardia* spp. The contamination rate of raw vegetables collected from the farm with 14% was higher than those collected from the market with 11.25%. It was concluded that the *Entamoeba* spp. was the most contamination in examined raw vegetables and the contamination rate of *Giardia* spp. and *Entamoeba* spp. in raw vegetables obtained from farm were higher than market. The findings can contribute baseline epidemiological information on intestinal protozoan contamination of fresh vegetables in Koya City and support improved hygiene practices in vegetable production, handling, transportation, marketing and consumption. Therefore, the health administration should provide a public education on prevention of fecal-oral transmission through eating fresh raw vegetables and the consumers also should properly wash and disinfect the raw vegetables before consumption.

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1. Introduction

Intestinal parasites are considered within the major Threats to the public health globally (1). A great Number of studies have been focusing recently on food-borne illnesses and their direct relationships with

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raw fresh vegetables consumption (2). Vegetables can be contaminated by various stages of parasites.

The most accepted theory is concluded that contamination of plants is happens while it is still in fields prior to harvesting, which can occur either via untreated wastewater from husbandry sources and or by direct way from animals, irrigation water from contaminated water supplies with sewage, or contaminated manure (3,4,5), or at the time of harvesting, transportation, handling and hygiene practices of preparation in food services, distribution and selling or even at home settings. Higher ratios of contamination are recorded in developing countries comparing to developed countries due to the unavailability of proper and solid system for regular diagnosis or reporting of a number of foodborne pathogenic agents, and therefore most outbreaks resulted from contaminated vegetables pass without adequate detection and underestimation of the range of their occurrence in food (6). Among the intestinal parasites that may cause serious problems to human as a consequence of consuming undercooked, contaminated or improper washing of vegetables are *Giardia intestinalis* and *Entamoeba histolytica* (7).

In the literature, there are some studies have been performed to evaluate the level of intestinal parasite transmission via raw vegetables, as in Tripoli, Libya (8), Riyadh, Saudi Arabia (2), different markets, Iraq (9), Tehran, Iran (10), and Manila, Philippines (11). The outcomes of these studies denote that the raw vegetables can be affected by various levels of parasitic contamination.

These protozoa can be a threat to the public health by consumption of raw or minimal processing vegetables and may serve as vehicles for transmission of intestinal protozoan parasites. Up to our knowledge, there is no previously published data about the contamination of freshly eaten leafy vegetables in Koya City which is one of the largest cities in the Erbil Province of Kurdistan Region –Iraq. However, comparative evidence on the occurrence of Giardia spp. and Entamoeba spp. in vegetables from different points of the food supply chain in Koya City in the Erbil Province of Kurdistan region- Iraq remains limited. Therefore, it can be necessary to do a study for detecting the parasitic protozoan in some common green leafy vegetables that are used in raw consumption in this area and the aim of this survey is to assess the contamination rate of common raw vegetables among markets and farms by the protozoan of *Giardia* spp. and *Entamoeba* spp. The novelty of this study lies in its direct comparative assessment of *Giardia* spp. and *Entamoeba* spp. contamination at two stages of the vegetable supply chain, farm and market, in Koya City. By comparing contamination before and after vegetables enter the retail environment, the study may provide locally relevant evidence about potential points of contamination and inform targeted food hygiene and public health interventions. This study can assist to health authorities with the exercise of control and finally promotion of public health. The study will also provide important information to stake holders on the possible contamination of vegetables.

2. Materials and Methods

2.1. General description of vegetable farms

There are 27 farms which they cultivate and supply the raw vegetables from Koya City in the Erbil Province of Kurdistan region – Iraq. These vegetable farms are irrigated by surface water in 7 farms, ground water in 4

farms and wastewater in 16 farms. Wastewater is the most common sources of irrigation in vegetable farms. 22 vegetable farms utilize the natural fertilizer. Also, animal excreta and human excreta are used in 17 and 5 vegetable farms, respectively.

2.2. Sample collection

Samples (400) from five types of common fresh vegetables (celery, parsley, leek, radish and green onion) were collected using simple random sampling in dates and seasons of spring (March, April and May months) and summer (June, July, and August months) and places of markets and farms. Each vegetable sample was placed in a separate nylon bag and labeled with a unique number and date of collection with labeling by season (spring and summer) and place (markets and farms). These samples were obtained from different markets and farms in the center and around of Koya city. 500 g from each vegetable was sampled in a clean plastic bag and they were sent to the microbiology and parasitology laboratory.

2.3. Sample examination

Samples including 200 g of each fresh vegetable were chopped into small pieces, then the sample put in a plastic bag and it was filled with 1 L of physiological saline solution (0.95% NaCl) and it was left out overnight. After that, 300 mL of the supernatant liquid was removed and the remainder part transferred to 5 mL test tubes and centrifuged at 2000 g for 20 min. Following centrifugation, the supernatant of all specimens was removed and the sediment was agitated gently by hand to redistribute the parasitic stages. The sediment was examined under a light microscope using × 10 and × 40 objectives for parasitic protozoa stages (12) after adding a drop of Lugol iodine (13). The parasites stages were identified according to Souls by procedure (14). The cysts for *Giardia* spp. and *Entamoeba* spp. in prepared slides were identified microscopically based on their characteristic morphological features, including size, shape, number and appearance of nuclei, cyst-wall characteristics, and internal structures. Finally, the cysts was confirmed by a specialist technician and the information was recorded.

2.4. Data analysis

Statistical analyses were carried out using Chi-square test of the SPSS software version 22 for windows (SPSS Inc., Chicago, IL, USA) to compare the rate of contamination of vegetables types from markets and farms. Descriptive statistical analysis and cross tabs were done. Chi-square and binary regressions with 95% confidence interval were done to find out statistical significance. p-value less than 0.05 was used to declare the significance association.

3. Results

A total of 400 fresh vegetable samples collected from farms and local markets. They were examined for parasitic examination, with particular emphasis on cysts of *Giardia* spp. and *Entamoeba* spp. The examination revealed the presence of parasitic contamination.

3.1. Protozoan parasites contamination rate

Protozoan parasites contamination in types of raw vegetables are shown in Table 1. Results showed that the most of the protozoa contamination of vegetables was from *Entamoeba* spp. cyst and totally, the frequency of this parasite was in 60 samples (15%). Parsley has the highest contamination among the vegetables.

Table 1. Contamination rate of raw green vegetables with cyst of *Giardia* spp. and *Entamoeba* spp.

Vegetable name /samples		*Giardia* spp.		*Entamoeba* spp.		Total contaminated	Prevalence
		N	Percent	N	Percent	N	Percent
Parsley	80	11	2.75	16	4.00	27	6.75
Celery	80	15	3.75	13	3.25	28	7.00
Leek	80	3	0.75	6	1.50	9	2.25
Radish	80	8	2.00	10	2.50	18	4.50
Green onion	80	4	1.00	15	3.75	19	4.75
Total	400	41	10.25	60	15.00	101	25.25

Table 2. Frequency of parasitic protozoan cysts in raw vegetable contamination according to collection place and season

Item	*Giardia spp.*		*Entamoeba spp.*		Total contaminated	Prevalence
	N	Percent N	Percent N	Percent		
Season						
Spring	18	4.50	27	6.75	45	11.25
Summer	23	5.75	33	8.25	56	14.00
Total contaminated	41	10.25	60	15.00	101	25.25
Collection place						
Farm	22	5.50	34	8.50	56	14.00
Market	19	4.75	26	6.50	45	11.25
Total contaminated	41	10.25	60	15	101	25.25

Table 3. Prevalence of contaminated and un contaminated vegetables by cyst of *Giardia* spp. and *Entamoeba* spp. in spring and summer seasons

		Season				
		Spring		Summer		
Protozoa	Contamination status N	Percent	N	Percent	X^2	p-value
Giardia spp.	Negative 182	91.00	177	88.50	0.670	.41
	Positive 18	9.00	23	11.50		
Entamoeba spp.	Negative 173	86.50	167	83.50	0.70	0.40
	Positive 27	13.50	33	16.50		

Table 4. Prevalence of contaminated and un contaminated vegetables by cysts of *Giardia* spp. and *Entamoeba* spp. according to collection places of farm and market

		Collection place				
		Market		Farm		
Protozoa	Contamination status N	Percent	N	Percent	X^2	p-value
Giardia spp.	Negative 181	90.50	178	89.00	0.250	.62
	Positive 19	9.50	22	11.00		
Entamoeba spp.	Negative 174	87.00	166	83.00	1.25	0.26
	Positive 26	13.00	34	17.00		

The contamination rate for parsley was 4% from *Entamoeba* spp. Similarly, leek, radish and green onion had the contamination from *Entamoeba* spp. with 1.5%, 2.5%, and 3.75%, respectively. But the highest contamination celery was from *Giardia* spp. with 3.75%. *Giardia* spp. contamination and type of vegetable had a statistically significant association (p=0.009).

3.2. Seasonal variation of contamination

Seasonal variation of protozoan contamination in raw vegetables has been shown in Table 2. The results showed that the contamination of raw vegetables was higher in the summer season than the spring. Also, the contamination of raw vegetables in summer season for *Giardia* spp. *and Entamoeba* spp. were 5.75% and 8.25%, respectively and there was not a statistically significant association. The total contamination level in summer season was higher than spring season. Also, the contamination rate of *Giardia* spp. and *Entamoeba* spp. in collected raw vegetables from farm were 5.50% and 8.50%, respectively and it was higher than those collected from the market.

The total contamination level of *Entamoeba* spp. was also higher for summer season 8.25% than spring season 6.75% (Table 2).

Table 3 shows the prevalence of contaminated and un contaminated vegetables by *Giardia* spp. and *Entamoeba* spp. in spring and summer seasons. There was not a statistically significant between *Giardia* spp. contamination in seasons of spring and summer (p ≥0.05). 91% and 88.5 % of vegetable samples were negative for *Giardia* spp. in spring and summer, respectively. Also, 86.5% and 83.5 % of vegetable samples were negative for *Entamoeba* spp. in spring and summer, respectively. It showed that there was not a statistically significant for *Entamoeba* spp. contamination (p ≥0.05).

3.3. Contamination of raw vegetables and collection places

The contamination rate of farm raw vegetables was 11 % and 17 % for cysts of *Giardia* spp. and *Entamoeba* spp, respectively (Table 4) and it shows that the contamination rate of raw vegetables collected from the farm was higher than those collected from the market. 90.5% and 89 % of vegetable samples were negative for *Giardia* spp. in market and farm, respectively. Also, 87% and 83 % of vegetable samples were negative for *Entamoeba* spp. in market and farm, respectively. There was not a statistically significant between *Giardia* spp. and *Entamoeba* spp. contamination in places of farm and market (p≥0.05).

4. Discussion

The utilization of wastewater for irrigation and natural fertilizer in agriculture can contaminate the plant products with microbial agents (15, 16). Vegetables like salad are not commonly cooked to preserve heat labile vitamins and the taste. These practices make raw vegetables a channel for transmission of protozoa infections. So, identifying the contamination of fresh raw vegetables has public health importance to target prevention of protozoa infections. The fecal-oral rout is an important transmission for causing the most of the parasitic infections like *Giardia* spp *and Entameoba* spp. (17).

In this study, celery (7%) and parsley (6.75%) were the most contaminated vegetables to *Giardia* spp. and *Entamoeba* spp., and also radish and green onion had the rate of contamination 4.5% and 4.75% respectively. The vegetable of Leek was the least contaminated

vegetable with 2.25%. Different contamination rates were identified by different studies.

In the present investigation, parsley, green onion, radish, celery and leek had the highest contamination from *Entameoba* spp. with the prevalence rate of 4%, 3.75%, 2.5%, 3.25 and 1.5%, respectively. Celery had highest contamination from cyst for *Giardia* spp. Leek had 0.75% of its contamination from *Giardia* spp. whereas similar studies identified 10% contamination from *Gardia* spp. in Iran (18). Generally, in this study, the contamination prevalence in vegetables was 15% for *Entamoeba* spp. and 10.25% for *Giardia* spp. The contamination rates from *Entamoeba* spp. from similar studies were 6.8% in Eygpt (19), 35.5% in Saud Arabia (20) and 37.5% in Gaza governorate (21). Similarly, the contamination rate from *Giardia* spp. was 8.8% in Egypt (19), 6.8% and 11% in Iran (22, 23), and 7.1% in Suleymani city, Kurdistan, Iraq (24). In addition, *Giardia* spp. have also been detected in commonly consumed raw vegetables in Egypt (25). Other reported contamination rates included 10% in Libya (8), 31.6% in Saudi Arabia (20), and 28.7% in Gaza Governorate (26). The observed differences might be due to differences in environmental conditions, the weather conditions of the study areas, agricultural practice, sanitary practice, and the sample size difference between the studies. The other most important contributor for the difference is the inclusion of all protozoa stages in similar studies while the current study concentrated on cyst and oocyst stages.

Also, this study presented that 14% of fresh vegetables tested in the summer and 11.25% of fresh vegetables tested in the spring were contaminated by protozoa. Similarly, the study from Egypt 49.0% in the summer and 26.9% in the spring had identified higher rate of contamination in summer season than spring season (19). The higher contamination in the summer than in spring might have association with the dryness of the summer season because the cysts will be easily washed out by the scanty rain in the spring than the dry summer.

In our research, the contamination of raw vegetables collected from places of farm and market were determined and it revealed that the raw vegetables from farm had higher contamination than market. Similarly, studies from Iran identified higher contamination for vegetables collected from farm than those collected from market (10, 23). The relatively higher percentage of contamination for vegetables collected from farms might be due to the higher utilization of waste water irrigation in the study area, personal hygiene of the farm workers and the lower literacy level of the farm workers where the majority of them had primary school or no formal education. The practice of washing before marketing to add market value on the vegetables might have reduced the percentage of contamination in the market.

At present study, although there was a visible percentage difference in the contamination of vegetables by season and type of vegetables, but there was not a statistically significant contribution for parasitic contamination. Similarly, a study from Egypt found no significant difference for contamination by seasonal variation (25). A study from Suleymani city in Kurdistan of Iraq had found no statistically significant difference between vegetable types and contamination (24). However, the place from where the vegetables were collected had a statistically significant association

with contamination. Vegetables from farm were more likely to be contaminated than vegetables from market. In the current study, vegetable type and *Giardia* spp. contamination had also a significant association. The most important factors which might contribute to the contamination of vegetables by protozoa in the study area were the use of waste water for irrigation by 59.3% of the farms and the utilization of natural fertilizers by 81.5% of the farms. These factors are identified as the sources of contaminants by various studies (15, 16, 26). At the present study, there are limitations in microscopic identification. It is acknowledged that the morphology-based microscopy method cannot reliably distinguish all *Entamoeba* species from all free-living amoebae. Therefore, in our study, the identification is interpreted as morphological identification rather than molecular confirmation.

5. Conclusion

It was indicated that the protozoan contamination in raw vegetables is high. The *Entamoeba* spp. was the most prevalent contaminant protozoa of raw vegetables. The contamination of raw vegetables collected from farm was higher than those were collected from market. Also, the rate of *Entamoeba* spp. and *Giardia* spp. contamination in summer was higher than spring. It needs to promote the vegetable safety and health administration should provide a public education on prevention of fecal-oral transmission through eating fresh raw vegetables. Also, this administration should take initiative to integrate the agricultural and health sectors to act jointly on the prevention of vegetable contamination. The farm workers and owners should be trained on the important aspects of contamination prevention and control. Trainings directed toward waste water management and appropriate use of natural fertilizers should be provided for the farm workers and farm owners. The consumers should properly wash raw vegetables before consumption. It needs some practice for a holistic approach in sustainable program to address this issue and present recommendations to overcome the causes of intestinal parasitic infections and remedy the problem completely.

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Author contributions

Study concept, design, and critical revision of the manuscript for important intellectual content: Hemn Sleman Ali, Sarmad Nagemaldeen Mageed, and Gholamreza Jahed Khaniki; drafting of the manuscript and consultation: Masud Yunesian, Nabi Shariatifar, and Mostafa Rezaeian; performing the experiments: Hemn Sleman Ali and Kochar Khasro Saleh**.**

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data are available on demand.

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