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PARASITIC CONTAMINATION OF TWO COMMONLY CONSUMED LEAFY VEGETABLES IN EL-KHARGA OASIS, UPPER EGYPT, AND EVALUATION OF HYGIENE PRACTICES AMONG THE VENDORS

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Abstract

Foodborne parasitic diseases cause human morbidity and mortality especially in the low- & middle-income countries. This study identified the parasitic contaminated *Eruca sativa* & *Raphanus sativus* cultivated in El-Kharga Oasis, Upper Egypt, as to seasonal variation and the vendors hygiene practices. A total of 270 samples of the two vegetables were purchased from public markets. Parasitic contamination was evaluated by sedimentation and flotation techniques with modified Ziehl-Neelsen stain. The results showed that 219(81%) samples were contaminated with 13 different parasites. *Ascaris lumbricoides* 123(25.5%) and *Cryptosporidium parvum* (14.9%) were the commonest ones and the highest contamination rate was in autumn (87%) for *E. sativa* and in winter (87%) for *R. sativus*. The contamination level was significant with the gender and washing practices of vendors. The current data gave evidence of the seasonal dynamics of acquiring parasitic infection due to consumption of raw vegetables, interaction, and its relevance with vendor's hygiene.

Keywords: El-Kharga Oasis, Food-borne parasites, Vendors, Health hygiene.

Introduction

Vegetables are an important source of dietary fiber, vitamins and minerals, have low energy density and provide a range of nutrients' required to regulate body's metabolic functions (Slavin and Lloyd, 2012). Thus, many people regularly consume fresh vegetables, which were sources of parasitosis associated contaminated fresh for sale vegetables (Hassan *et al,* 2012). But, apart from Egypt, disease outbreaks caused by the ingestion of contaminated vegetables and fruits pose a significant problem to human health in Saudi Arabia (Al-Megrin, 2010), in United States (Herman *et al*, 2015) in Turkey (Yeni *et al,* 2016), South Africa (Iwu and Okoh, 2019), UAR (El Bakri *et al,* 2020) and China (Li *et al,* 2020).

Generally, diarrhea (WHO, 2003), or food poisoning (Al-Agroudi *et al,* 2016) caused by raw vegetables or fruits consumption due to parasites, bacteria, or virus or residual insecticides (El-Bahnasawy *et al,* 2014), or irrigated with wastewater of man and animals (Al- Shawa and Mwafy, 2007), and/or farms (Daryani *et al*, 2008).

Generally, *Eruca sativa* oil and *Raphanus sativus* oil improve the glucose tolerance, & serum insulin level (Ahmed *et al*, 2016), but being risky when contaminated with parasites or microorganisms, insecticidal residue, bad transportation, industrial developm ent (Slifko *et al*, 2000), and/or marketing (Okyay *et al*, 2004).

The present study aimed to evaluate the parasitic prevalence and seasonal availability in two common leafy vegetables *Eruca sativa* (rocket) and *Raphanus sativus* (radish) in El-Kharga Oasis, Upper Egypt.

Methods and Materials

Descriptive study (cross-sectional) was conducted in the New Valley Governorate in El-Kharga Oasis in Upper Egypt, located between 22º 30' 14" & 26º 00' 00"N and between 30º 27' 00" & 30º 47' 00"E, from November 2018 to October 2019.

Ethical approval: All participants were voluntary and informed the work and that the results would be confidential. Sheet and written consent were obtained. Ethical approval was obtained from the Ethical Committee, Faculty of Medicine, Assuit University.

Data collection: A semi-structured questionnnaire was used to collect the information on parasitic risks contaminated with consumption of raw vegetables. A total of 54 vendors participated and the data were anonymous. The questionnaires were prepared in English and translated into Arabic language during the face to face interview. Questionnaire sheet included the sex, age and educational level, as well as knowledge about foodborne diseases and other factors that may be relevant for contamination as washing vegetables before display or not, freshly collected or preserved for more than 1 day, source of water used for washing. A total of 270 specimens were collected from different sales outlets. Vegetables (180 *E. sativa* & 90 *R. sativus*) were collected with the roots placed in separate labeled clean polyethylene bags without any manual contact and immediately transported for processing and examinations.

Parasites were eight protozoa and five helminthes stages. Parasitological examination was done (Takayanagui *et al,* 2007) with mild modification. The vegetables were washed by putting in tank filled with dechlorinated water for 3-4min to remove mud. Approximately 200g of each vegetable was soaked in a sterile glass container with 150ml of washing solution of phosphate-buffered saline (pH 7.4) and 0.01% Tween 80 (Sigma Aldrich, CAS no: 9005656) followed by vigorous shaking for 15min. The solution was then filtered through four-fold gauze, and dispensed into clean centrifuge tubes, and centrifuged at 2000×g for 30min. Supernatant was decanted into another tube and examined by zinc sulfate flotation method for cysts (El- Khabaz *et al*, 2019). Sediment pellet was mixed, and a simple smear was stained with iodine stain and microscopically examined for eggs, cysts, and larvae. Each sample was independently examined by low and high power magnifications as: a- a direct smear, in which a drop of sediment was applied on a clean slide and covered with a clean cover slip, b- an iodine smear, in which 0.1ml of sediment was mixed with a drop of Lugol's iodine on a clean slide, and c- sediment was stained with modified ZiehlNeelsen stain for *Cryptosporidium* oocysts (El-Naggar *et al*, 2006).

Statistical analysis: Data were analyzed by using SPSS software version 20 (IBM, Chicago, IL, USA), and summarized by descriptive statistics for infected parasites number and percentage (Mahmoud *et al*, 2020). Pearson's chi-squared test analyzed the prevalence of the examined vegetables during different seasons with total parasitic contamination and to correlate between socio-demographic and hygiene practices and vegetables contamination. *P* values ≤ 0.05 were considered significant. Ward's hierarchical cluster analysis was performed using PAST software to unify the groups so that the variation in these groups did not increase too drastically.

Results

The samples showed a high level of parasitic contamination in both the leafy vegetables with an overall prevalent rate of 219/270(81%). *E. sativa* showed a higher parasitic contamination level (85%) than *R sativus* (73%). Contaminated vegetables 22%, 12% & 47% showed single protozoan, single helminth, and mixed infections, respectively (Tab. 1), with mixed infection was significantly higher than a single infection either with protozoan or helminth ($P \leq 0.05$).

Table 1: Prevalence of intestinal parasitic infections in examined fresh leafy vegetables.

Vegetables	No	+ve	One protozoan No (%)	One helminth No (%)	Mixed Infection No (%)	Total protozoa No (%)	Total helminths No (%)
Eruca sativa	180	153 (85)	42 (23.3)	21 (11.7)	90 (33.3)	132 (73.3)	111 (61.7)
Raphanus sativus	90	66 (73.3)	18 (20)	12 (13.3)	36 (40)	54 (60)	48 (53.3)
Total	270	219 (81.1)	60 (22.2)	33 (12.2)	126 (46.7)	186 (68.9)	159 (58.9)

The frequency distribution of parasitic contamination on leafy vegetables (Tab.

2) showed *C. parvum* & *Cyclospora spp.* protozoa identified in 66(12%) and 54 (10%) vegetable samples, respectively. *Ascaris lumbricoides* (123, 22%) was the most frequently detected parasitic helminths, followed by *Fasciola* EMC (36, 7%).Twenty parasitic species were identified were the most prevalent and predominant fied (Figs. 1&2). Of the 20 parasitic species, 14 were detected in *R. sativus* during this survey. But, *Endolimax nana*, coccidian cysts or oocytes, *Trichocephalus trichiuris* eggs, *Toxocara canis* eggs and metacercariae were only on *E. sativa*.

Table 2: Recovered of parasites on examined leafy *Eruca sativa & Raphanus sativus*

Parasites	Total	*Eruca sativa*	*Raphanus sativus*	Total frequency %
Entamoeba histolytica/dispar	30	21	9	6.2
Endolimax nana	3	3	0	0.62
Giardia lamblia	36	24	12	7.4
Cryptosporidium parvum	72	45	27	14.9
Cyclospora sp.	54	36	18	11.1
Isospora sp.	12	9	3	2.5
Eimeria sp.	33	24	9	6.8
Blastocystis hominis	21	18	3	4.3
Ascaris lumbercoides	123	90	33	25.5
Trichocephalus trichiuris	24	24	0	4.9
Toxocara canis	15	9	6	3.1
Hymenolepis nana	15	12	3	3.1
Fasciola EMC	45	39	6	9.3
Total recovered parasites	483	387	135	100

Parasitic seasonal contamination showed that the highest rate was (78; 87%) in winter and the lowest one (66; 73%) was in autumn and none parasite was on *R. sativus* in spring and summer (Tab. 3). Parasitic contamination rate on *E. sativa* was higher in spring (87%) than in summer (80%). According to frequency of parasites, there was significant seasonal variation for *E. sativa* ($P \leq 0.05$) with the highest parasitic contamination in autumn, followed by spring, winter and then summer, respectively. But, *R. sativus* showed the highest parasitic contamination in winter than in autumn, without significant variation ($P \geq 0.05$). Also, there was significant variation between *E. sativa* and *R. sativa* in autumn season ($P \leq 0.05$), but not in winter (Tab. 4). The dendrogram of cluster analysis showed *A. lumbricoides, C. parvum, Cyclospora* sp., and *Fasciola* sp. EMC, the three major parasitic species in *E. sativa* and *R. sativus*. Each cluster contained species that were highly similar (Fig. 3). The dendrogram showed three different clusters: the first group in winter season was 67.5, the second group in autumn season was 60, and the third group was in spring and summer seasons were 22.5.

Table 3: Prevalence of parasitic contamination in examined vegetables seasonally.

Vegetables	Autumn		Winter		Spring		Summer	
	No.	No.+ve	No.	No.+ve	No.	No.+ve	No.	No.+ve
Eruca sativa	45	39(86.7%)	45	39(86.7%)	45	39(86.7%)	45	36(80%)
Raphanus sativus	45	27(60%)	45	39(86.7%)	0.0	0.0	0.0	0.0
Total	90	66(73.3%)	90	78(86.7%)	45	39(86.7%)	45	36(80%)

N= total number of samples examined; n= number of contaminated samples; %= percentage of infection

Table 4: Parasites recovered from plants on different seasons.

Vegetables	Autumn (n=243)	Winter (n=162)	Spring (n=93)	Summer (n=54)	X^2	*P* value
	No. (%)	No. (%)	No. (%)	No. (%)		
E. sativa	183(75.3%)	90(55.6%)	93 (100%)	54(100%)	86.229	<0.001**
R. sativus	60(24.7%)	72(44.4%)	0(0.0)	0(0.0)	1.091	0.296
Total	243	162	93	54		
X^2	62.259	2.000	-	-		
P value	<0.001**	0.157	-	-		

Socio-demographic, health and handling practices of vendors were given (Tab. 5). There were significant (P >0.05) differences between the contamination level on the vegetables based on sex, hand washing after use of toilet and washing hands during selling period over day. Mean and method of vegetables displayed positively increased contamination level ($P \leq 0.05$), but vegetab-les put on the floor was more contaminated. There were no significant ($P \leq 0.05$) differences between age, & education before display, source, and availability of water for washing, cleaning environment before and during selling) and contamination level. Majority of vendor's hygiene practices (washing vegetables claimed that they invited the sanitary officials for inspection positively affected contamination level ($P \leq 0.05$). But, venders' knowledge about food poisoning and vegetable-borne diseases did not affect contamination level ($P > 0.05$)

Table 5: Socio-demographic, hygiene and handling practices of vendors & vegetables contamination

Variable	Examined samples (270)	Positive samples (219)	X^2	*P* value
Male	135	130	4.268	0.039*
Female	135	89		
Age: 20-30years	118	90	1.402	0.705
30-40	68	50		
40-50	68	63		
Above 50	16	16		
Education				
No formal	169	139	0.040	0.842
Formal	101	80		
Washed before display				
Yes	101	89	0.532	0.466
No	169	130		
Means of display				
On floor	101	22	16.325	<0.001**
On table	169	97		
Source of water				
Tap water	253	209	0.694	0.405
Ground water	17	10		

P≤0.05= significant, and P≥0.05 =non-significant

Discussion

The present study showed that 219 out of 270 of vegetable samples were contaminated with different parasite species. This high level of parasitic contamination with overall prevalence 81% was in agreement with Ahmed *et al*. (2020) who reported that the overall prevalence of parasitic vegetable contamination was 84.1%. On the other hand, several Egyptian studies showed different results from the present one. Said (2012) in Alexandria reported 31.7%, Eraky *et al*, (2014) in Qualyobia Governorate reported (29.6%) and Etewa *et al.* (2017) in Sharkia Governorate found (39%) of vegetables were contaminated with different parasites.

The variations in the prevalence rates of parasitic contamination among different studies in Egypt may be attributed to the differences within the geographical location, climate, form of water used for irrigation, type and number of samples examined, methods used for the detection of the intestinal parasites, handling methods, and the hygienic practices followed by the vendors. The current study showed high variations within the parasitic contamination between *E. sativa* (85%) and *R sativus* that showed lower rate of parasitic contamination (73%). This difference in the rate could be related to differences in number of examined samples for each type as in our selected area *E. sativa* is available throughout the seasons, whereas *R. sativus* seeds are often plantonly in autumn and fall, but its growth is suspended during peak summer owing to high temperatures that may cause radishes to bolt thereby making them essentially useless (Stopforth *et al*, 2004). Therefore, no data on radish samples in summer and spring.

This finding agreed with that reported in Alexandria by Said (2012) and Sharkia by Etewa *et al* (2017). But, this finding disagreed with Hassan *et al* (2012) who found that the radishes showed the highest rate of parasitic contamination (50%) in Alexandria. In this study, *A. lumbricoides* eggs were the most frequent parasites that contaminated the examined vegetables (22% of Bkele *et al.* (2017) they showed that *A. lumbericoides* eggs were the most prominent parasites in raw vegetables. It was also detected in 20.3% of samples in Alexandria (Said, 2012). Al-Shawa and Mwafy (2007) reported that *Ascaris* eggs on vegetables indicated that human manure was used as fertilizers and food-handlers was infected and transmitted infection. Other helminth eggs such as *Trichocephalus trichiuris, Toxocara canis, Hymenolepis nana and Fasciola* EMC were detected in *E. sativa* samples. Presence of these parasites indicated the use of human manure as fertilizer, with possibility of vegetables contamination during handling. Etewa *et al.* (2017) in Sharkia found that *Fasciola* EMC were 1.4% on the vegetables, especially on watercress samples (4.2%).

Cryptosporidium parvum was the second frequently recovered parasitic contaminant (12%), and the highest number was detected in *E. sativa* (64%) than R. *sativus* (36%).

The present rates were higher than other Egyptian studies as it was 29.3% in Alexandria (Said, 2012), and 7.6% in Sharkia (Etewa *et al,* 2017). Abroad, in Ethiopia it was 7.6% (Alemu *et al*, 2019), in Norway, it was 4% on lettuce (Robertson and Gjerde, 2001). The present study more or less agreed with Ahmed *et al.* (2020) in Assiut (69.4%). *Cryptosporidium* oocysts strongly adhere to plants irrigated (Macarisin *et al*, 2010), canal water (Massoud *et al,* 2008), and *C. parvum* is notorious for its resistance to chlorine disinfection, a mainstay of water treatment, and zoonotic infections, arise from consuming contaminated food or water (Chalmers *et al,* 2019). Geological studies showed that the present study oasis had a water level significant drawdown (El Sabri and El Sheikh, 2009). Thus, the overuse of groundwater and reuse of irrigation water in agriculture increased the contamination of vegetables and fruits with coccidial infections. Other coccidian species such as *Cyclospora* oocysts represented 10% of all detected parasites. the entire number of the detected parasites). This agreed with Abougrain *et al*. (2010), & The different higher and lower contamination rates were 21.3% in Alexandria (Said, 2012), and 1.4% in Assiut (Ahmed *et al*. (2020). Abroad, it was 29.8% in Nepal (Sherchand *et al,* 2004), 1.8% in Peru (Ortega *et al.* (1997).

In the present study, cysts of *E. histolytica*, and *Blastocystis hominis* were detected in 6%, & 4.3%, respectively. But, *B hominis* varied among Egyptian authors between 10% (Zuel-Fakkar *et al,* 2011), 7.1% (Hassan *et al*, 2012), 6.8% (Eraky *et al,* 2014); 6.2% (Etewa *et al*, 2017), and 4.1% (Ahmed *et al,* 2020).

As to *Isospora* species, *I. belli* was found in one mentally retarded Egyptian patient in Cairo (Rifaat and Salem, 1963), and *I. belli* (9.7%) among immunosuppressed children in Minia Governorate (Abdel-Hafeez *et al,* 2012). *Eimeria* is a genus of apicomplex parasites that includes various species capable of causing the disease coccidiosis in animals (Fayner, 1980). In Egypt, *Eimeria* species have high economic importance affected domestic rabbits El-Shahawi *et al,* 2012), sheep and goats (Mohamaden *et al,* 2018), camels (Abbas *et al,* 2019) and birds (Atef *et al,* 2020). Outbreaks of Egyptian and Saudi *C. cayetanensis* infection were due to consumption of food and water contaminated by oocysts that survived both physical and chemical disinfectants (Hussein *et al,* 2018).

In the present study, sex of the vender, means of display, type of water used for washing before display were significantly associated with the parasitic contamination of vegetables. This agreed with Tefera et *al*. (2014) in Ethiopia and Mohamed *et al*. (2016) in Sudan. In the present study, no significant association was between parasitic contamination, and washing vegetables before display (P >0.05). Also, most vendors used tap water to wash the vegetables to reduce the rate of contamination, but this didn’t totally eliminate parasitic and microbial contamination (Fallah *et al*, 2012).

Conclusion

Generally, consumption of raw fruits and vegetables differentially predicted better health than the consumption of processed fruits and vegetables.

The high parasitic rate indicated the well washing of raw vegetables and fruits for human safety. Parasitosis need to be controlled at all stages of vegetables cultivation processing, manufacturing, shipping, and preparation in human consumptions mainly raw ones. People in affected areas should pay careful attention to hygiene.

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Explanation of figures

Fig. 1: a- cysts of *E. histolytica*, b- *Endolimax nana*, c- *Giardia lamblia*, d- *Cryptosporidium parvum* oocysts stained with Zielh Nelsen stain, e- *Isospora belli*, f-. *Eimeria* spp.sporulated oocyst, g- *Eimeria* unsporulated oocyst, h- *Blastocystis hominis*, and i- *Cyclospora cayetanensis.*

Fig. 2: a- *Ascaris lumbricoides* egg, b- *Toxocara canis* egg, and c- *Hymenolepis nana* egg.

Fig. 3: Dendogram showed clusters of parasites seasonal abundance.