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Parasitic contamination of some Vegetables in Sharkia Governorate, Egypt

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***ABSTRACT* : Water and vegetable parasitic contamination is a cosmopolitan problem that affects a great part of the world including Nile delta in Egypt. The most eminent causal parasites are nematode larvae and pathogenic protozoans such as *Cryptosporidium* spp., *Giardia* spp., coccidian oocysts and microspores. In this study, we sought to investigate the incidence/prevalence/abundance of parasitic contamination in vegetables irrigated by contaminated water at different places in Sharkia governorate, Egypt. We compared the infection incidence rate for these plants to those irrigated with clean fresh water. We found vegetables dill (*Anethum graveolens)* infected with *Ascaris* eggs with arate of infection (25%), *Giardia* cyst with a rate of (12%), and *Cryptosporidium* oocyst With a rate (10%), parsely (*Petroselinum crispum*) infected with *Trichuris* eggs with rate (14%), Nematode larvae with rate (6%), and *Blastocystis hominis* with rate (2%), lecttuce (*lactuca sativa*) infected with *Dipylidium caninum* with rate (7%), *Ascaris* eggs with rate (25%), *Giardia* cyst with rate (15%), green radish (*Raphanus sativus var. longipinnatus*) infected with *Trichuris* eggs with rate (12%), Nematode larvae with rate (2%), *Entamoeba* cyst with a rate of (10%), watercress (*Nasuritum officinale*) infected with *Ascaris* eggs with rate (20%), *Entamoeba* cyst with rate (8%), coriander (*Apiaceae* sp.) infected with *Dipylidium caninum* spp. with rate of infection (6%) , *Giardia* cyst with a rate (13%), Cucumber (*Cucumis sativus*) infected with *Ascaris* eggs with rate (9%), *Trichuris* eggs with rate (6%), *Nematode larvae* with rate (4%), Carrot (*Daucus carota* L) infected with Nematode larvae with rate of infection (8%), *Giardia* cyst with rate (14%), Tomatoes (*Lycopersicon esculentum* Mill) infected with *Ascaris* eggs with rate (11%), *Entamoeba* cyst with rate (8%) and Green Pepper infected with Nematode larvae with rate (5%), *Entamoeba* cyst with rate (9%). We conclude that eating vegetables irrigated with contaminated water may imply a high-impact risk for the livestock and the human population.**
***AIM*: The purpose of this study was to investigate the type and degree of contamination caused by parasites in regularly used raw vegetables and green fodder, collected from open fields in Egypt's East Nile Delta.**

***KEYWORDS*: Oocysts, Larvae, Flotation techniques, Nematode eggs, Sedemidation.**

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I. INTRODUCTION

Vegetables are important sources of dietary nutrients, as well as fiber and phytochemicals. Many vegetables are eaten raw or partially cooked. However, vegetables are perishable; as such they are at their best when fresh. In the subtropics, different vegetables are readily available but they are prone to fecal contamination due to the application of untreated human wastes on farmlands, or the use of unsafe water body for irrigation **(Yahaya and Bishop, 2022)** Parasitic and microbial Parasitic and microbial contamination and the pattern of occurrence of the parasite species depends on weather conditions, sociocultural conditions, sampling season, analyzed vegetable products, and other factors. Therefore, local assessment of vegetable contamination is crucial for targeted and effective interventions **(Asfaw *et al.*, 2023).** These infections share soil and food-borne transmission as major variables in their human population predominance because they discharge their eggs/ larvae in the feces of infected hosts, contaminating food or polluting soil due to unhealthy hygienic practices (**Chelkeba *et al.*, 2022)** Gastrointestinal diseases are abundantly seen in developing countries where contaminated water, poor sanitation, poor hygiene, and civil wars contribute to their prevalence which has a great impact on global health **(Mandeville *et al*., 2009)**. Studies all over the world have shown that many species of enteric helminths and protozoa can infect humans who consume contaminated, improperly washed fruits and vegetables **(Al-Shawa and Mwafy, 2007; Adanir and Tasci, 2013; Ismail, 2016)**. Intestinal parasites such as *Cryptosporidium* spp., *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, hookworms, *Enterobius vermicularis*, *Trichuris trichiura*, *Toxocara* spp., *Hymenolepis* spp., *Taenia* spp., *Fasciola* spp., and members of the family trichostrongylidae could infect humans as a result of consumption of contaminated, uncooked, or improperly washed vegetables **(Kozan *et al*., 2007)**.

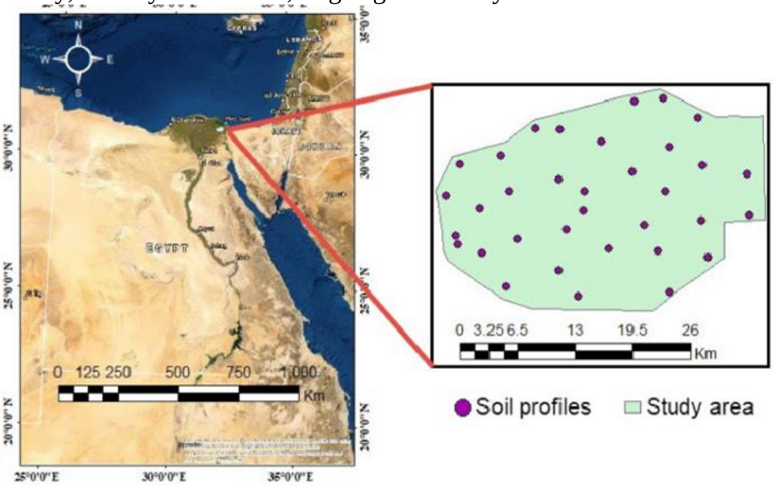
Given the increased demand for ready-to-eat foods, particularly those containing uncooked fresh vegetables, there is a great concern regarding the safety of these foods in the presence of unhygienic and improper management of raw produce. Successful intervention strategies are therefore reliant on identifying not only the practices that are important for consumer protection but also barriers that prevent consumers from responding to these interventions **(Fischer *et al*., 2006)**. Utilization of untreated organic fertilizer or use of contaminated water during the cultivation of vegetables in gardens or large-scale farms can lead to parasitic contamination. When such vegetables are finally consumed, they can lead to intestinal parasitic infections **(Al-Megrin, 2010; Faour-Klingbeil *et al*., 2016; Istifanus and Panda, 2018; Nyirenda *et al*., 2021)**. Some complications like weight loss, anemia , and deformities of the intestinal wall can also occur **(Bishop and Yohanna, 2018)**. Therefore, vegetables should always be cultivated, stored and distributed under safe and hygienic methods. They must be properly washed in running water, and/or cooked before consumption.

II. MATERIALS AND METHODS

Location of the study and sample collection

Vegetable were obtained from five different canals; El-Ganabia, El-Mahmodyia, El-Senety, Moias, and Saft Zerek canals. These canals are the source of irrigation for the nearby agricultural fields and farms in different agricultural areas in Sharkia.

Vegetables samples were collected from the nearby farms and fields irrigated by these canals. 400 from each of the following vegetables as (Table 1 and Fig. 3) were collected; **dill** (*Anethum graveolens*), **paresly** (*Petroselinum crispum*), **lecttuce** (*Lactuca* of *sativa*), **green radish** (*Raphanus sativus var. longipinnatus*), **Carrot (*Daucus carota* L)**, **watercress** (*Nasuritum officinale*), **Tomatoes (*Lycopersicon esculentum* Mill)**, **Green Pepper**, and **coriander** (*Apiaceae* sp.). All samples were transferred on the same day of collection to the Parasitology laboratory, Faculty of Science, Zagazig University.



Figs (1,2): Locations of the study area.

Detection of parasitic stages in irrigation water samples

Collected water samples were filtered through a stainless-steel pressure filter holder (Sartorius) using a nitrocellulose membrane filter (0.45 µm pore size and 142 mm diameter). The membrane filter was washed 3 times (each with sterile physiological saline). The water samples were sedimented by centrifuging at approximately 2000 rpm for 2 min. according to **Al-shawa and Mwafy, (2007**). Lugol's iodine-stained smears were prepared from the precipitate for light microscopy examination at different magnifications and photographed with a light microscope .

Detection of Parasitic stages on raw green leafy vegetables.

Vegetable samples were transmitted to a large clean plastic container and then washed twice, first with 2 L distilled water and then with 2 L detergent solution (**Luz *et al*., 2017)** with vigorous shaking for 15 min (**Al-shawa and Mwafy, 2007**). 100 g from each fresh vegetable was chopped into small pieces and put into a clean beaker containing engough physiological saline solution (0.85 NaCl) to wash and immerse the sample vegetable. Samples were kept overnight for sedimentation. The next day, the top layer of the washing saline was carefully discharged, and 5 ml of the sediment was centrifuged at 2000 rpm. After discarding the supernatant, the residue was mounted on slides, and stained in Lugol's iodine solution. Smears were examined using a light microscope at different magnifications. To increase the chance of parasitic detection, three slides were prepared for each sample.

Table (1): Name of the vegetable samples.

Common Name	Latin Name	Family
Lettuce	***Lactuca sativa L.***	***Asteraceae***
Green Radish	***Raphanus raphanistrum subsp. sativus (L.) Domin***	***Brassicaceae***
Carrot	***Daucus carota L.***	***Apiaceae***
Coriander	***Coriandrum sativum L.***	***Apiaceae***
Parsley	***Petroselinum crispum (Mill.) Fuss***	***Apiaceae***
Tomato	***Lycopersicon esculentum Mill.***	***Solanaceae***
Green Pepper	***Capsicum annuum L.***	***Solanaceae***
watercress	***Nasuritum officinale***	***Eruca vesicaria L.***
Dill	***(Anethum graveolens)***	***Anethum graveolens***

Figure 3-12 Vegetable species collected for current study

Fig 3 (*Lactuca sativa L.*)

Fig 4 (*Anethum graveolens*)

Fig 5 (*Petroselinum crispum (Mill.)*

Fig 6 (*Coriandrum sativum L.*)

Fig 7 (*Daucus carota L.*)

Fig 8 (*Lycopersicon esculentum Mill.*)

Fig 9 (*Nasuritum officinale*)

Fig 10 (*Cucumis sativus L.*)

Fig11 (*Raphanus raphanistrum subsp. sativus (L.)*

Fig 12 (*Capsicum annuum L.*)

III. RESULTS

Destribution of parasites in vegetables

Table (2) Shows the number and type of freshly eaten vegetable samples that were thoroughly inspected, as well as the proportion of contaminated samples. When the 400 vegetable samples were tested for *parasitic* contamination, 249 (62.25%) of them significantly tested positive. About 80% of the parsley samples proved positive for *parasites* infected with *Trichuris* spp. egg, *Nematode spp*. Larvae, and *Blastocystis hominis* spp among all plants with uneven surfaces. Dill samples had the lowest proportion of *parasitic* infected with *Ascaris spp*. Eggs, *Giardia spp*. cyst and *Cryptosporidium* spp. oocyst contamination (41.7%). Green pepper had the highest level of contamination (67.5%) infected with *Nematode spp*. larvae *Entamoeba* spp. cyst among vegetables with smooth surfaces, whereas cucumber samples had the lowest (50%) infected with *Ascaris* spp., *Trichuris* spp. egg, *Giardia spp*. In this investigation, 65.1% of the vegetable samples proved positive for one parasite species. Similarly, 25.7% and 9.2% of positive samples tested positive for two and three parasite species, respectively but a significant finding was recorded with the three parasitic species' contamination.

Table (2): The number and type of totally examined freshly eaten vegetable samples as well as the proportion of contaminated samples.

Type of vegetables	Item	Number of examined samples	Number of contaminated samples	% of contaminated samples	Polyparasitism Parasite Species Detected n (%)		
					One species	Two Species	Three species
Irregular surface	Coriander	50	31	62.0	22 (71.0)	7(22.6)	2 (6.5)
	Dill	50	25	41.7	20 (80.0)	4 (16.0)	1(4.0)
	Lettuce	40	27	67.5	17 (63.0)	7 (26.0)	3 (11.1)
	Radish	40	30	75.0	20 (66.7)	10 (33.3)	0 (0.0)
	Parsley	40	32	80.0	22 (68.8)	9 (28.1)	1 (3.1)
	Watercress	50	22	44.0	12 (54.5)	7 (31.9)	3 (13.6)
Irregular surface	Carrot	30	20	66.7	9 (45.0)	5 (25.0)	6 (30.0)
	Tomatoes	30	20	66.7	11 (55.0)	5 (25.0)	4 (20.0)
	Green Pepper	40	27	67.5	19 (70.3)	6 (22.2)	2 (7.4)
Total		400	249	62.25	162 (65.1)	64 (25.7)	23 (9.2)
Test			26.86		9.06	3.02	19.48
P			0.0001*		0.43NS	0.96NS	0.02*

***Significant P value < 0.05 NS= non-significant P value >0.05**

Several parasite species (helminths and protozoa) were isolated from the studied vegetable samples in this investigation with significant results (Table 2 and Fig. 13–22). Most Positive samples contained Ascaris species eggs, Trichuris species eggs, and Giardia species cysts, which were predominantly isolated from vegetables with uneven surfaces. The parasite Blastocystis hominis was the least common in positive vegetable samples. The examined vegetables showed negative results for some parasites eggs such as Ancylostoma spp.

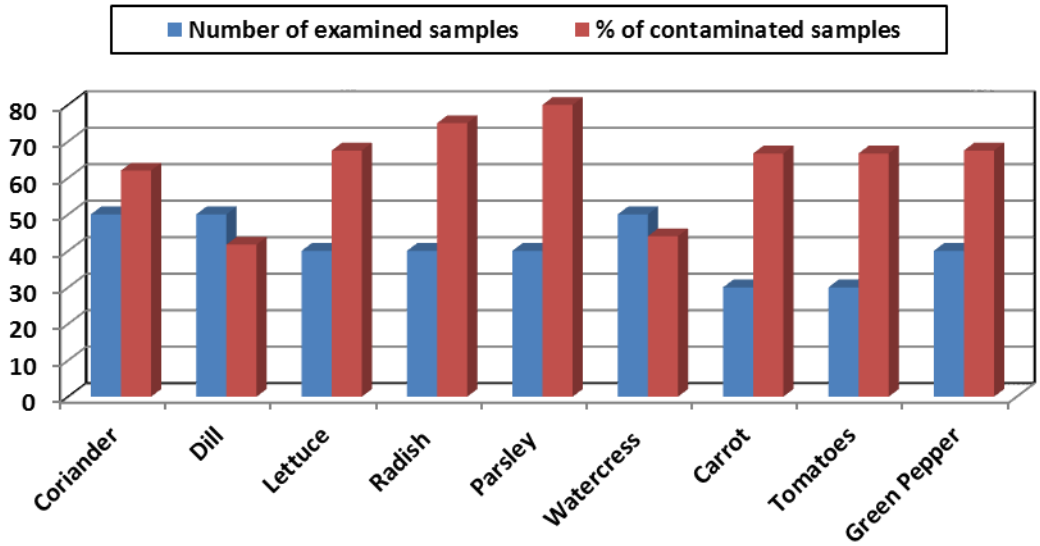


Fig. (13): Comparison between a number of examined samples from freshly eaten vegetables and % of contaminated samples.

Table (3): The percentage and type of parasitic species detected in the freshly eaten vegetables positive samples

Parasite	Number of positive samples (total= 249)	% of positive samples (%)	Coriander n =31	Dill n = 25	Lettuce n =27	Radish n = 30	Parsley n = 32	Watercress n =22	Carrot n = 20	Cucumber n = 15	Tomatoes n = 20	Green Pepper n = 27	P
Helminths													
***Ascaris* Eggs**	205	82.3	26	25	25	27	30	20	12	9	11	20	NS
***Trichuris* Egg**	100	40.2	10	11	11	12	14	14	8	6	9	5	NS
Nematode Larvae	55	22.1	10	7	0	2	6	9	8	4	4	5	NS
Dipylidium caninum	33	13.2	6	8	7	2	4	3	0	0	3	0	NS
Protozoa													
***Giardia* cyst**	104	41.8	13	12	15	7	10	10	14	9	6	8	NS
***Cryptosporidium* Oocyst**	66	26.5	7	10	8	5	9	10	4	4	1	8	NS
***Entamoeba* Cyst**	55	22.1	0	0	9	10	6	8	6	9	8	9	NS
***Balantidium coli* cyst**	45	18.2	5	6	6	0	8	3	7	5	0	5	NS
Blastocystis hominis	28	11.2	3	6	4	3	2	1	1	3	3	2	NS
P value	<0.001**												

**** Significant P value <0.05 NS= non-significant P value**

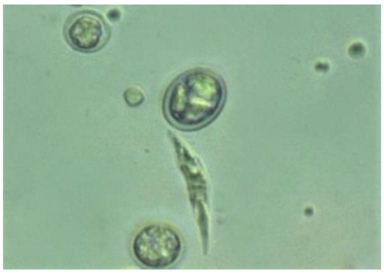


Fig. 14 (Entamoeba coli)

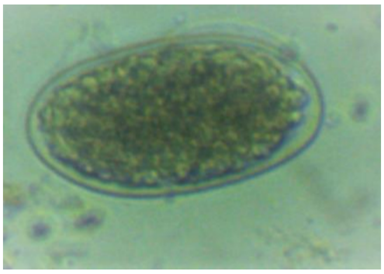


Fig .15 (Ascaris Lumbricoides)

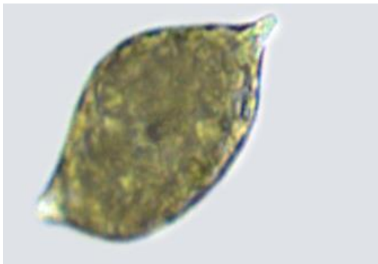


Fig.16(trichuris trichiura)

Fig. 17(Balantidium Coli cyst)

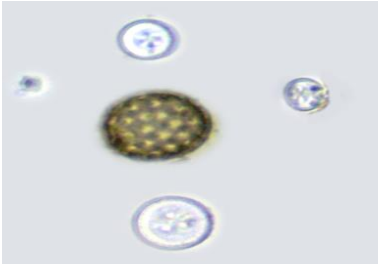


Fig .18 *(Dipylidium sp)*

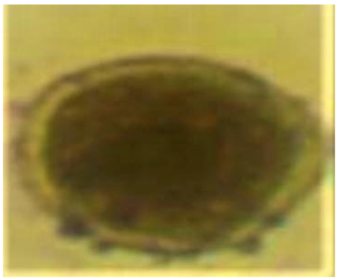


Fig 19 (Blastocystis sp)

Fig. 20 (Giardia Eggs)

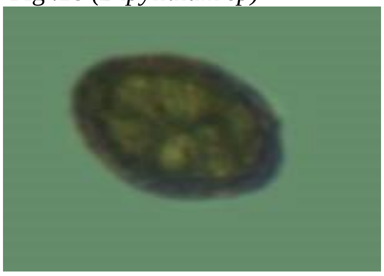


Fig. 21 (Giardia Eggs)

Fig.22 (nematode larvae)

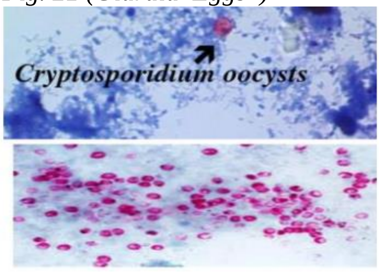


Fig .23 (Cryptosporidium spp oocyst (Z N) stained)

Fig. (14-23): Photomicrographs of fresh preparation smears showing parasites (×10), (×40) (100x)

IV. DISSCUCION

The isolation of medically important intestinal parasites from vegetables suggests that vegetables are potential sources of foodborne illness in humans. Their presence in the vegetables is associated not only with climatic conditions favorable to the existence and spread of parasites, but also with sanitary conditions and hygienic practices that facilitate their transmission. (**Asfaw *et al.*, 2023).** Certainly, irrigation with contaminated water became a painful reality in some villages in Sharkia governorate due to a lack of clean canal water, a result of water contamination by improper sewage disposal. Vegetables are very vital sources of nourishment. However, they may get contaminated by different forms and stages of intestinal parasites which are thought to affect about 3.5 billion people **(Wakid, 2009)**. In less developed countries, there is an increased chance of transmission of parasitic infections to vegetables during cultivation by sewage-contaminated irrigation water and untreated organic fertilizers carrying fecal helminthic eggs and larvae. Also, post-harvest handling, washing, transfer, and storage contribute to their contamination **(Pires *et al.*, 2012).** Consumption of raw vegetables, especially salads is almost a daily practice in Egypt. Thus, high incidence rates of intestinal parasitic infections have been reported in raw vegetable-consuming communities **(Fallah *et al.*, 2012).** Sharkia is a big agricultural governorate in Egypt that cultivates and its population consumes a variety of green vegetables. Fresh green vegetables, particularly those consumed raw in salad dishes or barely cooked, are a major source of intestinal parasite transmission and have been connected to food-borne parasitic infections in many communities throughout the world (**Chalmers *et al.*, 2020**, **Maia *et al.*, 2020**). Contamination most likely occurs before harvest while they are in fields, either by unregulated use of contaminated raw human or animal manure, sewage sludge, infected irrigation water, or directly through unsanitary hygienic practices of humans such as open defecation in farmlands. All of these probable contamination occurrences are feasible vectors of parasite transmission and compatible with the premise that the contamination level of soil and irrigation water is high in developing countries which has an impact on the safety of growing plants **Pires *et al.*, 2012**. The present study goes hand in hand with our result reported a significant difference in the number of Giardia cysts and Cryptosporidium oocysts positive samples detected using different diagnostic techniques in each season as well as in the total number of positive samples detected throughout the year. These results could be attributed to global warming which stimulates parasite growth or to the use of highly sensitive techniques for testing. It also could be related to where the source of samples, as Delta is highly infiltrated with farms with extensive animal breeding activities. Lastly, it could be caused by the high resistance of these parasites to the usually used water disinfectants such as chlorine attributed to their strong (oo)cyst walls **(Zahedi *et al*., 2021)** Because these parasites employ a broad variety of transmission pathways, the life cycle phases found in food and used as detection targets vary substantially. In the 400 samples of green vegetables examined, nine parasites from the metazoa and protozoa groups were identified. Metazoa (helminths) included Ascaris spp., Nematode spp. larvae, Trichuris spp., and Dipylidium caninum, while protozoa included Blastocystis spp., Balantidium spp., Entamoeba spp., Giardia spp., Cryptosporidium spp. These parasite entities are emphasized as key causal factors of infection conveyed by vegetable-derived meals (**FAO, 2016).** Other studies from different parts of the world showed a lower rate of contamination such as 32.6 in Iran [Ardabil] (**Daryani *et al*., 2008)**. For example, lower rates of contamination have been shown in Ardabil, Iran (32.6%) (**Daryani *et al*., 2008)**, in Saudi Arabia (16.4%) (**Al-Megrin, 2010)**. On the other hand, other researchers have show higher levels of contamination compared to our study; 71% in Iran (Shahrekord) (**Fallah *et al*., 2011);** 58% in Libya (**Abougrain *et al*., 2010)**; and 75.9% in Kenya (**Nyarango *et al.*, 2008)**. The observed discrepancies can be attributed to variations in geographical location, the size and type of sampling, parasitic infection rate of hosts in each area, techniques employed for sampling and even the agricultural methods in different regions. Different laboratory procedures may also play a role in retrieving different parasites since some procedures can either float or sediment the parasites.

The high level of contamination in these samples is probably due to the utilization of high amounts of animal manures. It should be mentioned that in the majority of the examined samples, animal manure adhered to vegetables was observed. More importantly, untreated sewage as fertilizer for the cultivation of vegetables should be avoided. Furthermore, preventing animals from entering the vegetable farms, washing, cleaning and disinfecting the vegetables especially before raw consumption, education, sanitation and treatment of infected people in Soran can significantly decline the rate of contamination.

V. Conclusion and Recommendations

The results of this study demonstrate that contamination of fresh vegetables with parasites, bacteria and fungus is a public health risk. Similarly, some socio-demographic characteristics, as well as hygiene and safety conditions, were found to be significantly related to the parasitic contamination of vegetables. According to this study, the microbiological count of vegetables sold in Debre Berhan town is influenced by the time of the day. The microbial count of vegetables increases as the day advances, being lower in the morning and higher in the afternoon. Therefore, home consumers should go to the market in the mornings or early in the day to buy vegetables. In general, the results suggest that food safety practices in place by various actors along the food supply chain, from farming practices of farmers to handling practices of food retailers and distributors are generally poor. Food manufacturers, retailers, and distributors should minimize the risk of contamination of fresh vegetables with parasites, bacteria, and fungus to ensure acceptable Crops and soil irrigated with fresh water were free from parasites eggs and cysts. The occurrence of pathogens in soil and crops implies health risks for farms, produce handlers and consumers.

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