Scientific Journal for the Faculty of Science-Sirte University
Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index
DOI: 10.37375/issn.2789-858X

Parasite Contamination of Fresh Leafy Vegetables in Benghazi, Libya.

Entesar Salem Elmnefi[1 *], Najat Idrees Alshibli[1], Najwa Mahmoud Alfurjani[1], Heba Amrajaa Salh[1], Sami Fadlallah Altom[1], Mohammad Ali Faraj[1], Fawzia Ali Al- Majbri[2].

[1]*Department Of Medical Technology. College of Sciences and Technology[*] Gaminis , Benghazi , Libya.*

[2] *Depertment Parasitology, Referencial Medical laboratory of Benghazi, Benghazi, Libya.*

DOI: https://doi.org/10.37375/sjfssu.v4i2.2893

ARTICLE INFO:

Received: 01 August 2024

Accepted:02 October 2024

Published: 26 October 2024

Keywords: Vegetables, Helminths, Parasitology, parasitic diseases, contamination, Libya

A B S T R A C T

Raw vegetable consumption can be a primary pathway for the spread of foodborne parasite illnesses, despite the fact that fresh vegetables offer significant nutritional value to people. Thus. This study's goal was to investigate whether parasites are present in veggies that are frequently eaten in Benghazi city, Libya. Method:- of the study One hundred and fifteen types of vegetables. lettuce, mint, parsley, green onion, and watercress 30 pieces of each were included. The sediments experienced analysis using iodine and modified smears stained with Ziehl- Neelsen, while data SPSS version 25 statistical software was used for the analysis. Results:- It was discovered that (90%) of the samples that were inspected contained parasite *eggs*, *cysts*, and *larvae*. With a contamination percentage of (100%), lettuce was the most contaminated vegetable, and parsley had the lowest contamination rate (80%). The parasite with the highest prevalence rate was *Entamoeba histolytica/ dispar cyst* (71.1%), *Giardia Lamblia cyst* and *Hymenolepis nana eggs* (13.3%)each of them, *Taenia. Saginata eggs* (11.11%), *Ascaris eggs* and *Entamoeba. Hartmanni cyst* (6.67%) each of them, but it was discovered that the *Strongyloides larvae* only occasionally appeared (6.67%).
Conclusion:- Benghazi City leafy vegetables had a very high parasitic rate of contamination. Therefore, eating such vegetables without thoroughly cleaning them promotes the spread of parasitic diseases, which provide a serious risk to health for those in Libya.

1. Introduction.

Fresh vegetables are an important part of a healthy diet because they include important vitamins, minerals, and dietary fibers. Additionally, they have phytochemical components that exhibit antioxidant action, for example, to retain their tastes, many vegetables are often eaten raw or very little cooked toimprove the natural taste and keep heat - sensitive

nutrients would be stable. The intake of raw vegetables can act as a major conduit for the transmission of several foodborne diseases because of their porous and complex surface. Unfortunately, vegetables may facilitate the adhesion and survival of infections. (LIU,,2003). Throughout the life cycle of vegetable production, this might happen at a number of points, including field cultivation, harvesting, handling, distribution, marketing, and even consumer consumption(Alegbeleye et al.,2018),(Iwu and Okoh., 2019). An area's level of contamination can be impacted by a variety of factors, such as using filthy

Corresponding author:- E-mail: *Entesarsalemal@gmail.com*

water for irrigation and using untreated manure as fertilizer which have some antibacterial properties as well as antiviral and anticarcinogenic properties (Garedaghi et al.,2011). Animal waste from both domestic and wild sources, as well as human waste, post-harvest handling, and hygienic preparation conditions in homes or food service settings can be done too.(Murray et al.,2017),(Ulukanligil et al.,2011).Consuming fresh vegetables has been linked to a rise in food-borne disease cases that have been documented recently(Erdogrul and Sener.,2005).

Consuming vegetables that are usually undercooked, uncooked, or inadequately prepared increases the risk of contracting parasite infections. Vegetables can transmit *protozoa cysts* and *oocytsts* that cause human illness, including *Giardia*, *Entamoeba*, *Toxoplasma*, *Cryptosporidium*, and *Isospora*, as well as helminthe eggs and larvae, such as *Ascaris*, *Toxocara*, *Hymenolepis*, *Taenia*, *Fasciola*, *Enterobius*, *Trichuris*, *Trichostrogylus*, *Strongyloides*, and *hookworms*(Beuchat et al.,2002),(Kozan.,2007).

It may be dangerous for human health to consume infected vegetables. To help the appropriate authorities take the appropriate steps to promote food safety and protect public health, information on contamination of fresh vegetables is necessary.

2. Materials and Methods.

2.1 Study area.

This study was conducted in Benghazi which is the second largest city in Libya overlooking the coast of the mediterranean sea and is located at latitude 32°" 11′ 67 north and longitude 20°" 06′ 67 east and the population is about 2,340,673 people according to the population census in 2012, and weather moderately hot summers and mild winters(Wikipedia.,2009).

2. 2 Sample collection.

This study was conducted during the period from December 2023 to February 2024.

The study included 150 samples of vegetables consisting of five types of fresh leafy vegetables 30 samples of each type:-(lettuce/ watercress / green onions / Mint /Parsley) 200 g for each type, these veggies were chosen for the study because they are common & often eaten raw and sold in all of the study area's markets. A random sample of markets in Benghazi and farms was also chosen to collect data.

Afterward, every sample was carefully placed in an individual plastic sterile bag and labeled with a specific identifier, such as a unique number, sample name, collection place, or collection date, within 24 hours of collection, the samples were then transported to the microbiology laboratory where they underwent thorough processing and examination for the detection of parasites.

2.3. Sample preparation and Examination.

200 g of each type of vegetable were soaked in 500 ml of physiological saline solution (0.95% sodium chloride), and the mixture was mixed for 15 minutes. This allowed each vegetable sample to be separated from the parasites. Each vegetable sample was then placed in a separate plastic sterile bag and labeled with a unique number, sample name, collection place, and collection date. The washing solution was left to stand on the bench for the full night in order to get the best sedimentation. The supernatant was evacuated the next day, and any big debris was removed by filtering the remaining wash solution through sterile gauze. It was then left to settle for an additional hour, after which the supernatant was disposed of once more. Conical tubes labeled "plain tube" held the leftover sediment, which was then centrifuged for 15 minutes at 3000 rpm to concentrate the parasitic stages. After the sediments

were mixed, each sample was checked for the presence of parasite ova and cysts.

Direct Smear: A drop of the sediment was placed in the center of a clean slide using 10X and 40X objectives, and a cover slide was carefully placed on to avoid air bubbles and spilling. A light microscope was then used to examine the preparation. Iodine smear: A drop of the sediment was mixed with a drop of Lugol's Iodine solution and examined similarly to a direct smear in order to identify parasite eggs, cysts, and larvae. The slides are then taken out and examined under a light microscope with 10X and 40X lenses(Alshareef et al.,2019).

2.4-Statistical Analysis.

The data and lab findings were arranged and assessed using the statistical package for social science (SPSS) version 25 computer application. Categorical data were compared between groups using the Chi Square Test ($\chi 2$ value) or the Fisher Exact Test (FET), and the Z test was used to assess the significance of the difference in the statistical comparison between the groups. A statistically significant p-value was defined as less than 0.05.

3. Results.

Table (1) as shown in the results of the study, (135 / 150) it showed the studied vegetable samples were contaminated with various parasites. *Protozoa*, *Helminths*, and the more contaminated type is Lettuce pollution 100 % (30 / 30), with an equal percentage of 90 % (27 / 30) for wach of the Mint, Green onion, and Watercress, while Parsley was 80% (24 / 30) loss polluted.

(Table 1):- Parasite contamination rate in fresh vegetables in Benghazi, Libya.

	Lettuce (30)		**Mint (30)**		**Green onion (30)**		**Watercress (30)**		**Parsley (30)**		**Total (150)**	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Positive	30	100 %	27	90%	27	90%	27	90%	24	80%	135/150	90 %
Negative	0	0	3	10%	3	10%	3	10%	6	20%	15/150	10 %
Z Test	14.78		10.19		10.19		10.19		13.21			
P value	0.002		0.001		0.001		0.001		0.17			

P - value < 0.05

Table (2) Vegetable contamination was identified in (135 to 150) fresh vegetables samples, they were contaminated with one type of parasite (Single Contamination) or mixed contamination (Double / triple Contamination) whereas single contamination had the higher percentage at (77.78%), followed by the Double Contamination of the vegetables samples for (20%) and the lowest percentage rate of contaminations(2.22%) was for samples which contains triple Contamination.

(Table 2):- Shows types of parasitical contaminations of vegetables studied in Benghazi, Libya.

		No. of contaminated samples	Single Contamination No. (%)	No. of mixed contamination		Total NO. (%)
				Double contamination	Triple cont.	
Valid	Lettuce	30	27	3	0	3 (10)
	Mint	27	21	6	0	6 (22.2)
	Green onion	27	18	9	0	9 (33.3)
	Watercress	27	24	3	0	3 (11.11)
	Parsley	24	15	6	3	9 (37.5)
	Total	135	105 (77.78%)	27 (20%)	3 (2.22%)	30 (22.22)

P - value < 0.048

Table (3) showed detected 98 type of parasites (73%) of which are protozoan cysts, that is the highest rate of contamination. The samples showed: *helminthic eggs*, *larvae* that appeared in the percentage of 37 samples with percentage of (27 %).

(Table 3):- percentage of Contamination of parasites types (*Protozoa*, *Helminths*) with fresh vegetables samples studied.

	The Type of pollution	Frequency	Percent
Valid	Protozoa Contamination No. (%)	98	73 %
	Helminths Contamination No. (%)	37	27%
	Total	135	100 %

P - value < 0.048

Table (4) the study showed the highest prevalent parasitic stage detected in the samples of raw vegetables, that present of *E. Histolytica / E.dispara Cyst,* the highest percentage in watercress samples was (28.12%), and the lowest one in mint samples that was (15.62%), an equal percentage was shown in Lettuce samples, green onion and parsley (18.75%). As for *H.Nana* was presented with percentage of (13.3%) and had the highest presence in mint samples with a rate of (66.67%) and had the lowest presence in green onion at a rate of (33.33%), and it was NIL in the Lettuce vegetable samples, Watercress and parsley. As for *Giardia Lamblia cyst*'s contamination rate was (13.3%), and it was (50%) in parsley samples which are more contaminated, and followed by rate of (33.33%) of the mint samples. The lowest percentage was found in salad samples which is (16.67%), and it was not found (absent) in green onion and watercress samples.

T. Saginata eggs has a pollution rate of (11.11%), and the highest pollution was in the samples of the Lettuce which was (80%), and the lowest pollution in the parsley samples was (20%), and it was not recorded in any of the samples of mint, green onion and watercress.

Ascaris eggs has a contamination rate of (6.67%) and its presence was recorded at (66.67%) in green onion, and at (33.33%) in parsley, and no presence was recorded in samples of Lettuce vegetables, mint and watercress.

E. Hartmanni trophozoite cyst has a contamination rate of (6.67%) and its presence was recorded in only two types of vegetable samples, with equal proportions of (50%) in both watercress and parsley. No presence was recorded in the samples of Lettuce vegetables, mint, and green onions. *Strongyloides Larva* has a contamination rate of (6.67%) and its presence was record-

ed only in green onion samples at a rate of (100%), and no presence was recorded in the other vegetable samples were studied.

(Table 4):- Distribution of parasitic contamination of fresh leafy vegetables in Benghazi, Libya.

Parasite	Number	% of Total Examined Samples (150)	% of Total Positive Samples (135)	Studied Vegetables					Total %
				Lettuce Number of +ve % 	Mint Number of +ve % 	Green onion Number of +ve % 	Watercress Number of +ve % 	Parsley Number of +ve % 	
E. Histolytica / E.dispara Cyst 	96	64 %	(71.1%)	18 (18.75%)	15 (15.62%)	18 (18.75%)	27 (28.12%)	18 (18.75%)	100 %
H. Nana eggs 	18	12 %	(13.3%)	0	12 (66.67%)	6 (33.33%)	0	0	100 %
Giardia Lamblia cyst 	18	12 %	(13.3%)	3 (16.67%)	6 (33.33%)	0	0	9 (50%)	100 %
T. Saginata eggs 	15	10 %	(11.11%)	12 (80%)	0	0	0	3 (20%)	100 %
Ascaris eggs 	9	6 %	(6.67%)	0	0	6 (66.67%)	0	3 (33.33%)	100 %
E. Hartmanni trophozoite cyst 	6	4 %	(6.67%)	0	0	0	3 (50%)	3 (50%)	100 %
Strongyloides Larva 	6	4 %	(6.67%)	0	0	6 (100%)	0	0	100 %
	X^2= 4.37	*P* - value < 0.048							

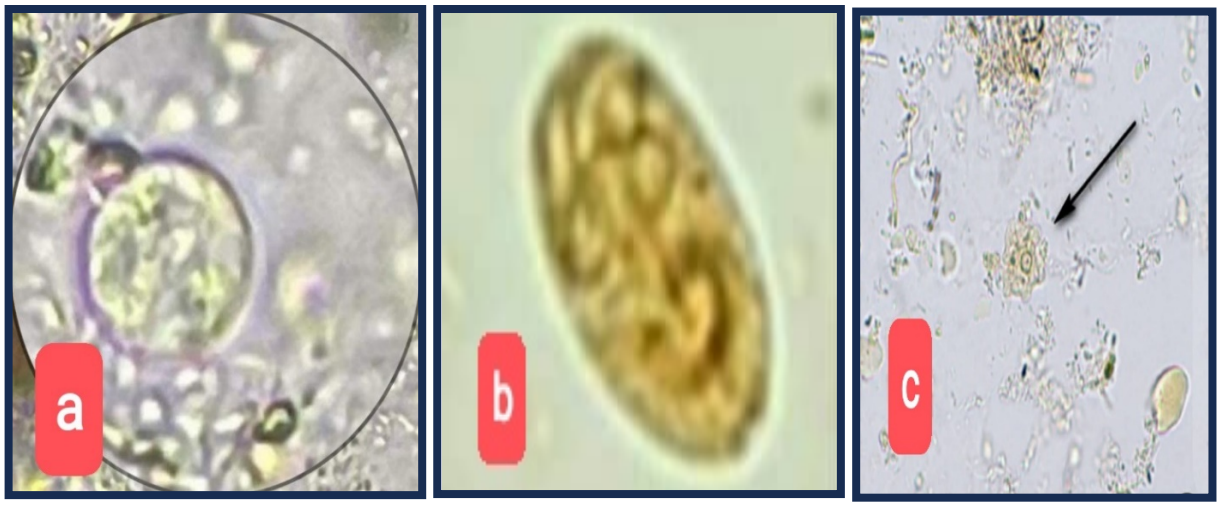

Figure 1: Shows the detected *protozoan* (*cysts/oocysts*) in the sample of vegetables have been studied from Benghazi, Libya: (a): *Entamoeba histolytica/dispar* cyst; (b): *Giardia lamblia* cyst; (c): *Entamoeba Hartmanni trophozoite* cyst.

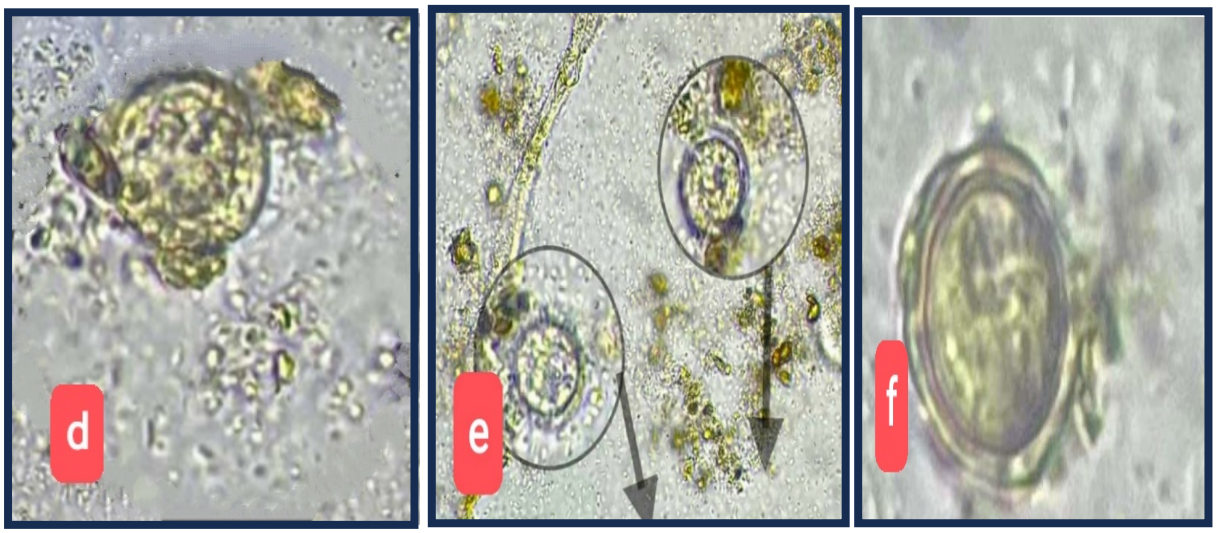

Figure 2: Shows the detected *Helminths* (eggs), in the sample of vegetables have been studied from Benghazi, Libya: (d): *Hymenolepis nana* egg; (*e*)*: Taenia Saginata eggs*; (*f*)*: Ascaris* egg.

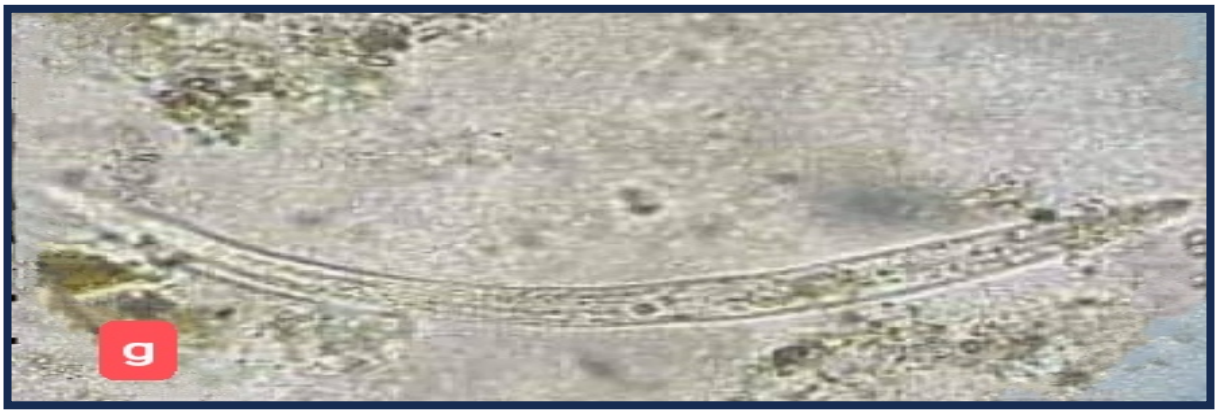

Figure 3: Shows the detected *Helminths* (*larva*), in the sample of vegetables have been studied from Benghazi, Libya: (g): *Strongyloides larva*.

3 Discussion.

One of the most important forms of nutrition is vegetables; the World Health Organization (WHO) recommended consuming 400 g or more of them daily to prevent chronic diseases and as a nutrient supplement(World Health Organization.,2003). However, veggies may contain intestinal parasites. Ar.ound 3.5 billion individuals worldwide are afflicted with intestinal parasites, the majority of people are infected are people who eat raw vegetables which is the main media of infections transmission (Wikipedia .,2009). Communities that eat raw vegetables have been found to have high prevalence of intestinal parasites, particularly if the crops are grown at farms that use untreated human and animal fertilizers(Srikanth et al.,2004). During vegetable growing, untreated organic fertilizers containing fecal helminthes and irrigation water tainted with sewage might facilitate the spread of parasitic illnesses.

Additionally, handling, washing, transferring, and storing them after harvest all of these contribute to the contamination of eggs and larvae (Pires et al.2012), (Torrence.,2016). Research aimed at identifying parasite contamination in vegetables which is spread globally, as the possibility of food-borne parasitic infection epidemics becomes more apparent (Said.,2012) Therefore, in order to determine the degree of parasite infection of a few regularly consumed vegetables, our study was conducted. In Libya, very few research have been done to assess the level of parasite infestation on vegetables.(Alshareef et al.,2019). Eating fresh vegetables that come from farms and are offered for sale at Benghazi City's wholesale and retail marketplaces. Vegetables contaminated with intestinal parasites can reveal information about the frequency of intestinal parasites in a certain population and are an indicator of animal or human fecal contamination(Al-Megrain .,2010).The overall rate of parasite infection was determined to be (90.0%), which was matched with earlier findings from Egypt (84.1%) and Yemen (100%) (Muhammad andRadman.,2019), (Ahmed et al.,2020),(El-Sayed et al.,2023). However, some investigations found lower contamination rates, including 5.9% in Turkey (Kozan et al.,2007). (16.2%) in Saudi Arabia (Al-Megrain .,2010) and (13.5%) in Sudan (Mohamed et al.,2016). Multiple variables may be responsible for the variations seen between this research and others. These rates might be changed, depends on, the region, the climate, the environment, the sample size, the methods, the postharvest treatment strategy, and the socioeconomic status (Abougrain et al.,2010),(Bekele et al.,2017). The rate of protozoan parasite contamination in this study was (73%), which was greater than the rate of *helminthes* parasite contamination (27%). This is consistent with the results of (Elmajdoub et al.,2017) who found that the Contamination rate for *protozoan cysts* was (79.4%) and (47.6%) for *helminthes* parasites. This could result in widespread intestinal parasite infections among humans (Youssef.,2014).

This study revealed a very high degree of intestinal parasite contamination in green leafy vegetables, which showed: lettuce has the greatest contamination (100%), followed by watercress (90%), green onions (90%), mint (90%), parsley (80%). The most contaminated crop was lettuce (30/30) (100%), Similar findings were published in a study conducted in Riyadh, Saudi Arabia, where lettuce (78.8%) and watercress (22.8%) had the greatest rates of infection, respectively(Al-Megrain.,2010). Samples of watercress and lettuce in Tripoli, Libya, were found to be more polluted than other samples, with contamination percentages of (100%) and (96%), respectively (Abougrain et al.,2010) , Lettuce (45.5%) and watercress (41.3%) exhibited the greatest levels of parasite infestation in Banha City, Egypt(Eraky et.al.,2014). Additionally, a study conducted in Saudi Arabia in 2006 found that green on-

ions had a higher percentage of infection (40%) than lettuce (28%), in Nigeria, the rates of contamination for watercress and lettuce were lower (17%) (Abougrainet al.,2006). This might be because of the amount of impurity changes which depends on the vegetable's surface and form. Due to their uneven surfaces, green leafy plants like lettuce, watercress, mint, and parsley are more susceptible to parasite cysts and eggs that are more attached to that kind of surface. Furthermore, during civilization, the inflexibility of vegetable leaves may facilitate interaction with the soil and, consequently, with *helminthes* structures that are likely established in the soil (Damen et al.,2007). These findings contradicted a number of other investigations that found the highest prevalence of contamination in lettuce and/or watercress (Said.,2007), (Abougrain et al.,2010).(Eraky et al.,2014). In this investigation, cysts of *Giardia lamblia* and *Entamoeba spp*. were found in (13.3%) and (71.1%), respectively, of the vegetable samples. This impurity is most likely caused by the high survivability of oocysts and excrescencies in the environment, as well as their propensity to attach explosively to the surfaces of plants, especially leafy greens like parsley and lettuce (Dorris et al.,2002). While there are several ways that make vegetables might get polluted, the most common ones are before harvest, when they are contaminated by sewage sludge, infected manure, irrigation water, or direct animal contamination from both domestic and wild animals (Said .,2007).The parasites that contaminated vegetable samples most frequently, in this investigation, (37.75%) of the samples had *helminthes eggs* or *larvae* found in them. It is commonly recognized that several of these species may parasitize animals such as cattle, dogs, and cats in addition to humans. In (Dorris et al.,2002) , *H. nana eggs* were found with (13.3%) of the samples that were analyzed; this percentage was similar to what (Said .,2007) reported, and (2.6%) of the vegetable samples in Alexandria, Egypt included *H. nana* (Fallah et al.,2012) ,(El-Sayed et al.,2023) .In a previous study conducted in Libya (Elmajdoubet al.,2017), (7.9%) of the vegetable samples contained *H. nana eggs*. However, other research revealed higher In Thousern Ethiopia's Arba Minch village, *H. nana eggs* were found in (15.56 %) of the vegetable samples that were analyzed. (Bekeleet al.,2017) and *H. nana eggs* were only found in (0.5%) of the green vegetables affected by the parasite in Qazvin, Iran, a lower rate was found there (AL-Sanabani et al.,2016). The observed discrepancy might be a result of variations in the geographic location and climate (Adejayan. And Morenikeji.,2015). Vegetables infected with *Taenia saginata* represent the least amount of contamination (11.11%). Research done at Amol, in the north of Iran (10%) (Siyadatpanah et al.,2013) Iran, Zahidan (13.2%) Manglore (11.9%)(Ebrahimzadeh et al.,2013).(Dias et al.,2014) Saudi Arabia, Tabuk 2.17% (Gabre and Shakir.,2016) Similar contamination rates were observed in Lahore, Pakistan (5.12%) (Maqbool et al.,2014) and the current study's findings. *Ascaris spp. eggs* (6.67%) were the last common contamination discovered in this study (Abougrain et al.,2010) , which indicated that *Ascaris spp*. was the most common intestinal parasite infecting salad in Tripoli, Libya, where vegetables are sold at wholesale and retail marketplaces. Additionally, *Ascaris spp*. *eggs* were the most commonly isolated parasites in vegetables in studies conducted in Saudi Arabia (Abougrain et al.,2006) , Iran (Damen et al.,2002), and Nigeria (Adejayan. And Morenikej .,2006).This dominance may be attributed to the parasite's widespread distribution and the resistant nature of the eggs, which allows them to survive unfavorable conditions. The eggs are resistant to desiccation for two to three weeks, may live for two years at 5 to 10 °C, and can even survive in the absence of oxygen (Robertson L.J. et al.,2000).Vegetables may contain *Ascaris eggs* and *nematode larvae* due to improper watering practices and possible usage of untreated manure. According to

Turkish research, *helminthes* transmitted through soil, primarily *Ascaris eggs*, were found in (14%) of fresh vegetables and (84%) of soil samples were taken vegetable farms using (61%) irrigation water (Ulukanligil et al .,2001). When we were at farms' sites, we noticed that most of the farmers kept their cats without fences, which might have exposed the crops to contamination from both domestic and wild animals, and that they utilized untreated animal dung as fertilizer for their crops. Animal manure is a well-known source of human pathogens that may live in soil for long periods of time. As a result, contaminant structures may be transmitted to produce and stay there for an extended period of time when used as soil fertilizer(Luz et al.,2017).

3 Conclusions.

This study's findings unequivocally demonstrate that raw vegetables are frequently tainted with parasites when ingested by humans. Consuming vegetables that have been infected with pathogenic parasites without properly washing and /or preparing them might be harmful to one's health. The best method for lowering food-borne parasite infection rates is still contamination prevention. Farmers, sellers, and the general public should all get thorough health education on the dangers of eating infected vegetables and the significance of cleaning and disinfecting them before eating. Therefore, it is strongly advised that control measures be implemented, including those relating to irrigation water quality criteria, prohibit domestic and wild animals from accessing plant farms, and refrain from utilizing untreated manure as fertilizer. Moreover, additional research on the parasite contamination of fruits, vegetables, and the land and water used to grow them is strongly advised. It is also recommended that these studies must be carried out in various parts of the country.

Conflict of interest.

There are no conflicts of interest, according to the writers.

References.

Abougrain A. M, Bello C.S, El-Shewy K, Abdulla S.E. (2006). The prevalence of parasites in commonly used leafy vegetables in South Western, Saudi Arabia. Saudi Medical Journal. Vol 27: 613- 616.

Abougrain A. K, Nahaisi M. H, Madi N. S, Saied M. M, and Ghenghesh K. S, (2010). Parasitological contamination in salad vegetables in Tripoli-Libya, Food Control, vol. 21, no. 5, pp. 760– 762.

Al-Megrain W. A. I. (2010). Prevalence of intestinal parasites in leafy vegetables in Riyadh, Saudi Arabia. International Journal of Zoological Research, vol 6(3):190-195.

Adejayan. A, Morenikeji O, (2015). Prevalence of Intestinal parasites in vegetables sold in major markets in Ibadan city, south-west Nigeria. Global journal of pure and applied sciences. vol 21:7- 12.

AL-Sanabani, A. W, Algalil, F. M.A, Radman, B. A. & AL-Manusorl, R. T, (2016). Prevalence of intestinal parasites in fresh leafy vegetables in some farms at Dhamar city, Yemen. Prevalence.

Alegbeleye O. O, Singleton I, Sant'Ana A. S, (2018). Sources and contamination routes of microbialpathogens to fresh produce during field cultivation: A review. Food Microbiol. Google Scholar[CrossRef]. vol 73, 177–208.

Alshareef S.A, Abdulsalam A.M, Alghanaei R. A, Salim E. S, Chibani M. (2019). Parasitic Contamination of raw vegetables sampled from different farm locations in Brack Al-Shati, Libya, Journal of Pure & Applied Sciences, Vol.18 No. 4.

Ahmed A.A, Sayed F.G, Galal L.A, Ismail T, Gaber M.M, (2020). Detection of parasites contaminating raw consumable vegetables in Assiut city, Assiut governorate, Egypt. Journal of the Egyptian Society of Parasitology, vol 50: 557-564.

Beuchat LR, (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables, Microbes Infect. vol 4(4):413-23. PubMed.

Bekele F, Tefera T, Biresaw G, Yohannes T. (2017). Parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, southern Ethiopia. Infectious Diseases of Poverty. vol 6: 19.

Dorris, M. Viney, E. and Blaxter, M. (2002). Molecular phylogenetic analysis of the genus Strongyloides and related nematodes. Internatinal Journal of Parasitology, vol 32(12): 1507-1517.

Damen J. G, Banwat E. B, Egah D. Z., and Allanana J. A, (2007). Parasitic contamination of vegetables in Jos, Nigeria, Annals of African Medicine, vol 6, no 3, pp 115–118.

Dias M, George M, and Dias M.R. (2014). How safe are vegetables parasitological contamination of fresh leafy vegetables in Mangalore, International Journal of Applied Biology and Pharmaceutical Technology, vol 5(4): 80-82.

Erdogrul O, Sener H. (2005). The contamination of various fruit and vegetables with Enterobius vermicularis, Ascaris eggs, Entamoeba histolytica cysts and Giardia lamblia cysts. Journal Food control, vol 16:527-560.

Ebrahimzadeh. A, Jamshidi. A, & Mohammadi. S, (2013). The parasitic contamination of raw vegetables consumed: Zahedan, Iran. Health Scope, vol :(4)1 ؛ 209-205 Doi: 10.5812 /jhs.8209.

Eraky M. A, Rashed S. M, Nasr M. E, El-Hamshary A. M. S, El-Ghannam A. S, (2014). Parasitic Contamination of Commonly Consumed Fresh Leafy Vegetables in Benha, Egypt. Journal of Parasitology Research,

Elmajdoub L.O, Omar M.A, Al-Aboudy M.S, Abosheba F, Elzwawi S, (2017). Prevalence of parasitic contamination of leafy green vegetables in Misurata, Libya Russian Journal of parasitology. vol 40: 197- 204.

El-Sayed. N. M, Gawdat, S. S, El-Kholy. H.S, Elmosalamy. A. (2023). Parasitic Contamination in Five Leafy Vegetables Collected from Open Market places in Giza, Egypt, Journal of Food Quality and Hazards Control 10 vol 13-20.

Fallah, A.A., K. Pirali-Kheirabadi, F. Shirvani,and S.S. Saei-Dehkordi, (2012). Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Food Control*, vol 25(2): 617-620.

GABRE, R.M. and SHAKIR, A., (2016). Prevalence of some human enteroparasites in commonly consumed raw vegetables in Tabuk, Saudi Arabia. Journal of Food Protection, vol. 79, no. 4, pp. 655-658. http://dx.doi.org/10.4315/0362-028X.JFP-15-485. PMid:27052871.

Garedaghi Y, Hashemzade F, & Pooryagoobi S. (2011). Parasitic contamination of fresh Vegetables consumed in Tabriz,Iran. Reasearch Tournal of Biological Sciences. vol 6(10):518-522.

Iwu C.D, Okoh A.I. (2019). Preharvest Transmission Routes of Fresh Produce Associated Bacterial Pathogens with Outbreak Potentials. A Review. Int. Journal Environ Res Public Health Google Scholar [Cross Ref][Green Version]. vol 16, 4407.

Kozan E, Sevimi F. K, Kose M, Eserm M, and Cicek H, (2007). Examination of helminth contaminated wastewaters used for agricultural purposes in Afyonkarahisar, Turkish Journal of Parasitology, vol. 31(3), pp. 197–200. PubMed.

Luz, J., M. Barbosa, A. de Carvalho, S. Resende, J. Dias, and H. Martins, (2017). Contamination by intestinal parasites in vegetables marketed in an area of Jequitinhonha Valley, Minas Gerais, Brazil. *Revista De Nutricao-Brazilian Journal of Nutrition*, vol 30(1): 127-136.

LIU, R.H, (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals, The American Journal of Clinical Nutrition, vol 78, no. 3, pp.517S-520S, Doi PMid:12936943.

Maqbool, A., Khan, U.J., Yasmin, G. and Sultana, R., (2014). Parasitic contamination of vegetables eaten raw in Lahore. Pakistan Journal of Zoology, vol. 46, no. 5, pp. 217-223.

Murray K, Wu, F, Shi J, Jun Xue, S, Warriner K. (2017). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. Food Qual. Saf. Vol 1, 289–301.

Muhammad T. A. A, Radman B.A. M, (2019). Prevalence of intestinal protozoan parasites in leafy vegetables Fresh in the markets of Radaa city-Al Baydha, Albaydha University Journal, vol (1)– Issue 2- December

Mohamed, M.A., E.E. Siddig, A.H. Elaagip, A.M.M. Edris, and A.A. Nasr, (2016). Parasitic contamination of fresh vegetables sold at central markets in Khartoum State, Sudan. *Annals of Clinical Microbiology and Antimicrobials*, vol 15(17). DOI 10.1186/s12941-016-0133-5.

Pires S. M, Vieira A. R, Perez E, Lo F, Wong D, Hald T. (2012). Attributing human food borne illness to food sources and water in Latin America and the Caribbean using data from outbreak investigations. Int J Food Microbial; vol 152(3):129 – 38.

Robertson L.J. et al. (2000). Isolation and enumeration of Giardia cysts, Cryptosporidium oocysts, and Ascaris eggs front fruits and vegetables. Journal of Food Protection.

Srikanth R, and Naik. D, (2004). Health effects of wastewater reuse for agriculture in the suburbs of Asmara City, Eritrea, International Journal of Occupational and Environmental Health, vol. 10, no. 3, pp. 284–288.

Siyadatpanah.A, Tabatabaei. F, Emami Zeydi. A, Spotin. A, Faliah-Omrani. V, Assadi. M, Moradi. S, Rostami. A, Memari. F, and Hajialiani. F (2013). Parasitic contamination of raw vegetables in Amol, North of Iran. Archives of Clinical Infectious Diseases, vol. 8,no.2,pp.1-4. http://dx.doi.org/10.5812/archcid.15983.

Said D. E, (2012). Detection of parasites in commonly consumed raw vegetables, Alexandria Journal of Medicine, vol. 48, pp. 345–352.

Torrence M. E, (2016). Introduction to Preharvest Food Safety. Microbiol. Spectr. [Google Scholar] [CrossRef] [PubMed]. Vol 4.

Ulukanligil, M., A. Seyrek, G. Aslan, H. Ozbilge, and S. Atay, (2001). Environmental pollution with soil-transmitted helminthes in Sanliurfa. Turkey, *Mem I Oswal Cruz*, vol 96: 903-909.

Wikipedia H. M. (2009). Improvement of Ritchie technique by identifying the food that can be consumed pre-analysis. J App Sci Res, vol 5:293-296.

World Health Organization, (2003). Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint FAO/WHO Expert Consultation, Vol. 916.

Youssef A. I, Uga S. (2014). Review of parasitic zoonoses in Egypt. Tropical Medicine and Health., vol 42: 3-14.