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Contamination of Fresh Vegetables by Parasites in Jardinah Region, Benghazi

Najla Abdul-Razig Najem
Department of Microbiology, Higher Institute of Science and Technology-Seluk, Benghazi, Libya.

Abstract

Eating raw vegetables is a significant way to spread various infectious diseases. This study aimed to investigate parasitic contamination in some commonly used salad vegetables in the Jardina region of Libya. The extraction process involves isolating parasites from fresh, raw vegetable samples to obtain analyzable samples for detection. Samples are concentrated using centrifugation, a process that separates heavier particles (such as parasitic stages) from the liquid, making it easier to analyze the sample and increasing the likelihood of detecting parasites. The concentrated samples are then stained using iodine stain and modified Ziehl-Neelsen stain. Finally, the samples are examined under a microscope. Of the 200 samples analyzed, 45% (90/200) contained parasites. The parasites included *Giardia lamblia* cysts, *Entamoeba histolytica* cysts, Entamoeba coli cysts, *Enterobius vermicularis* eggs, *Hymenolepis nana* eggs, *Ascaris lumbricoides* eggs, & *Cryptosporidium* spp. oocysts. The highest number of contaminated samples was found in coriander, 75%, while the lowest amount of contamination was found in tomatoes, 31.2%. The prevalence of parasites was highest for *G. lamblia* cysts in raw vegetable salads (15%). This was followed by *E. histolytica* cysts (10%), E. coli cysts (7%), 5% for each of *E. vermicularis* & *H. nana* eggs. *A. lumbricoides* eggs and *Cryptosporidium* spp. oocysts each accounted for 3%. These findings highlight the potential significance of these results for global food safety. The importance of raw vegetables used daily in salads lies in their potential to endanger public health through the transmission of intestinal parasites among people in the Jardina region.

Keywords: Vegetables, Parasite, contamination, Jardina, Libya

Introduction

Vegetables are a crucial part of a balanced diet, providing a rich source of vitamins and minerals. Regular consumption of vegetables is linked to a reduced risk of cancer, stroke, and cardiovascular diseases. They can help lower the risk of various health issues, including cancer, diabetes, Alzheimer's disease, cataracts, and heart disease. Dietitians recommend including vegetables in daily meals due to their substantial benefits for health improvement and disease prevention (1, 2).

On the other hand, fruits and vegetables, particularly when eaten raw and unpeeled, can transmit various parasites linked to infection outbreaks. Many vegetables are consumed raw or lightly cooked to retain their natural flavor and heat-sensitive nutrients. However, the consumption of raw vegetables poses a significant risk for foodborne illnesses, as their complex surfaces and porous structures facilitate the adherence and survival of pathogens (3, 4). Parasitic factors can contaminate vegetables in several ways:

1- The use of human fertilizers in agriculture, which may be contaminated with parasites such as Giardia, Cryptosporidium, Ascaris.

2- The application of animal fertilizers that can carry parasites common to both humans and domesticated animals.

3- The use of sewage for irrigation on farms.

4- Contamination of vegetables during various stages, including production, collection, transportation, and preparation for sale. (5)

Studies show a significant link between the consumption of vegetables, particularly raw ones, and the incidence of parasitic infections (6, 7, 8, 9). Many studies, including those conducted in Benha, Egypt (10), Benghazi, (11), Kufra (12) & Tripoli, Libya (13), Riyadh, Saudi Arabia (14), Mosul, Iraq (15) and Tehran, Iran (16). Have shown varying degrees of parasitic contamination in raw vegetables.

Intestinal parasitic infections can be contracted through various means, such as eating contaminated vegetables and water (17). In many developing countries, use of insufficiently treated wastewater to irrigate vegetables has been reported to be responsible for the high rates of contamination with pathogenic parasites (18). Consuming raw or undercooked vegetables is one way that intestinal parasitic infections can be spread (19). There are two possible sources of contaminated water: humans and animals. Infected animals can contaminate agricultural practices through techniques like slurry spraying or by handling, transporting, and storing their products in contaminated areas like farmyards. This risk could be increased by the growing interest in organic farming. Furthermore, there is a chance of contamination from those working as pickers and handlers or in other production and processing related roles (20). Epidemiological research has shown that in regions where parasitic diseases are prevalent and wastewater is utilized for irrigating raw vegetables, consuming such vegetables without adequate washing can result in parasitic infections (21). The analysis of 167 multistate outbreaks in the USA from 2006 to 2021 confirmed these observations. The etiological agents linked to these outbreaks were identified, with the primary sources attributed to cross-

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contamination in the distribution chain, inadequate agricultural practices, and fresh imports (22)

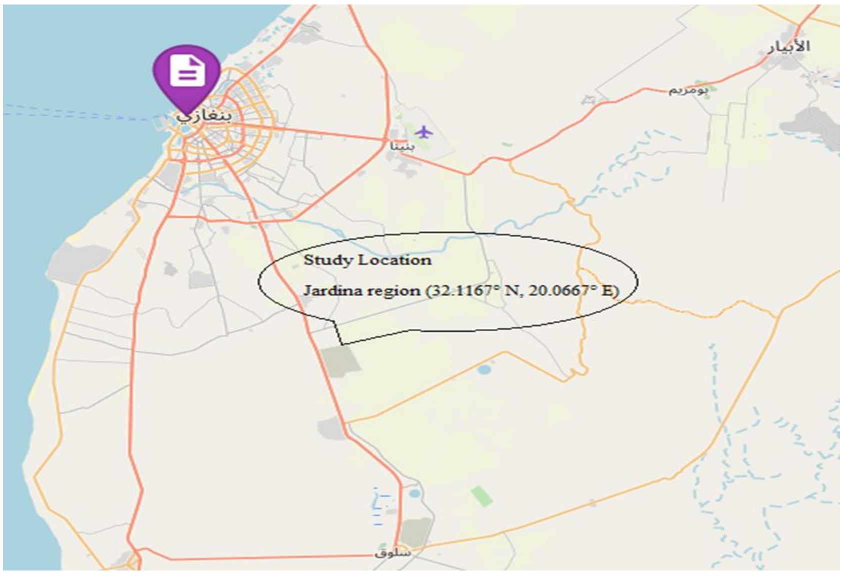
Many developing countries lack effective diagnostic and surveillance systems. As a result, regular checks of food are not conducted consistently, leading to contamination is not being detected in a timely manner (23).

This study focuses on several fresh vegetables, including coriander, parsley, tomatoes, green onions, and cucumbers, sourced from local markets in the Jardinah region. This area is renowned for its rich agricultural environment, with agriculture being one of the primary economic activities. A variety of crops, such as vegetables and fruits, are cultivated here, making it an important hub for agricultural production. These vegetables were selected due to their significance as essential components of the daily diet in Libya. Currently, there is no published data regarding the contamination of fresh leafy vegetables in the Jardinah region. Therefore, this study aims to provide stakeholders with valuable insights into the potential contamination of these vegetables.

Materials and methods

Study Location

The study was conducted from April 2023 to February 2024 in the Jardina region (32.1167° N, 20.0667° E), located southwest of Benghazi, Libya (as shown on the map). Vegetables are displayed in traditional Libyan markets allowing buyers to select their preferred quantities. Alternatively, there are stores specifically dedicated to selling vegetables, where these products are attractively arranged. Consumers also have the option to purchase vegetables directly from farmers, ensuring they receive fresh produce.



Sample Gathering

Random samples were taken from various stores located in the Jardina area. A total of 200 samples of seven different types of fresh vegetables were randomly selected for the study. The vegetables included varieties such as coriander (20), parsley (28), tomatoes (32), green onions (28), capsicums (35), cucumbers (30) and dill (27). 200 g of samples was taken from each type under normal purchasing conditions. The samples were placed in plastic bags, numbered, and sent to the microbiology laboratory at the Higher Institute of Science and Technology - Suluq for testing.

Samples preparation

Wash vegetables with half a liter of distilled water, then it is filtered with gauze to get rid of plant remains, such as the damaged stem or leaves, then leave for 10 hours until the sedimentation process is complete. The top layer was discarded and the remaining sediment wash water was transferred to a centrifuge tube to remove unwanted materials. The tube was centrifuged at 2000 rpm for 5 minutes for focused ova, larvae, cysts, and oocysts (7, 24). The supernatant, it is the liquid obtained after allowing a mixture to settle, as the denser materials collect at the bottom. The top liquid is carefully poured off to avoid mixing with sediment.

The hand was used to gently agitate the sediment, which helped distribute the parasites evenly before performing the analysis. Then the sediment was studied by placing a drop of this sediment on the slide and examining it under a microscope.

Samples examination

Direct smear: place a drop of the precipitate on the slide, then place the slide cover. Tilt the glass cover at an angle of 45 degrees, then gently lower it onto the sample. This technology ensures even fluid distribution, reducing air trapping underneath. The slide is placed on the stage to select the sample; start with the low (10X) magnification lens and then change the lens to the magnification (40X). A light microscope was subsequently employed to examine the preparation.

Iodine smear: A drop of the sediment was combined with a drop of Lugol's iodine solution and examined in the same manner as a direct smear to identify parasite eggs, cysts, and larvae (25).

The Modified Ziehl-Neelsen acid-fast stain was used to stain the oocysts of coccidian protozoa in the smear.

Result:

A total of 200 fresh salad vegetable samples were analyzed to check for parasitic contamination. Of the 200 samples that were analyzed, 45% (90/200) had parasites. The highest percentage of contaminated samples was found in coriander (75%), followed by green onions (53.5%), dill (51.8%), cucumbers (43.3%), and parsley (42.8%). In contrast, the lowest percentages of contamination were observed in capsicums (31.4%) and tomatoes (31.2%), as shown in Table 1.

Table 1: Parasitic contamination of fresh vegetables collected from various stores located in the Jardina region.

Vegetable	No. Examined	No. positive (%)
Coriander	20	15 (75)
Green onions	28	15 (53.5)
Dill	27	14 (51.8)
Cucumbers	30	13 (43.3)
Parsley	28	12 (42.8)
Capsicums	35	11 (31.4)
Tomatoes	32	10 (31.2)
Total	200	90 (45)

The parasites identified in the vegetable samples included *Giardia lamblia* cysts (15%), *Entamoeba histolytica* cysts (10%), *Entamoeba coli* cysts (7%), *Enterobius vermicularis* eggs (5%), *Hymenolepis nana* eggs (5%), *Ascaris lumbricoides* eggs (3%), and *Cryptosporidium* oocysts (3%). The analysis revealed that the presence of *G. lamblia* cysts was higher than that of other protozoan cysts/oocysts, which collectively accounted for 15%. The study indicated that *G. lamblia* and *E. histolytica* cysts were the most prevalent in the samples, followed by *E. coli* cysts, *E. vermicularis* eggs, *H. nana* eggs, *A. lumbricoides* eggs, and *Cryptosporidium* oocysts, as displayed in Table 2.

Table 2: Parasites found in collected vegetables from various stores in the Jardina region.

Parasite	No. positive (%)
Giardia lamblia cysts	30 (15)
Entamoeba histolytica cysts	20 (10)
Entamoeba coli	14 (7)
Enterobius vermicularis	10 (5)
Hymenolepis nana	10 (5)
Ascaris lumbricoides	3 (1.5)
Cryptosporidium	3 (1.5)

Table 3: Distribution of intestinal parasites across the vegetable types included in the study.

Vegetable	No. positive	*E. histolytica*	*G. lamblia*	*E. coli*	*Cryptosporidium spp.*	*H. nana*	*A. lumbricoides*	*E. vermicularis*
		Cyst %	Cyst %	Cyst	Oocysts %	Egg%		
Coriander	15	4 (26.6)	6 (40)	3 (20)	1 (6.6)	0	1 (6.6)	0
Parsley	12	3 (25)	2 (16.6)	1 (6.6)	0	2 (16.6)	1 (8.3)	3 (25)
Tomatoes	10	3 (30)	5 (50)	0	0	1 (10)	0	1 (10)
Green onions	15	2 (13.3)	4 (26.6)	4 (26.6)	2 (13.3)	3 (20)	0	0
Capsicums	11	2 (18.1)	6 (54.5)	0	0	2 (18.1)	0	1 (9.0)
Cucumbers	13	2 (15.3)	3 (23.0)	4 (30.7)	0	1 (7.6)	1 (7.6)	2 (15.3)
Dill	14	4 (28.5)	4 (28.5)	2 (14.2)	0	1 (7.1)	0	3 (21.4)
Total	90	20	30	14	3	10	3	10

Discussion

Libyan cuisine is characterized by its close relationship with fresh vegetables, which play an essential role in many traditional dishes. A wide variety of vegetables, such as tomatoes, green onions, coriander, parsley, cucumbers, capsicums, and dill, are used extensively. Most of the vegetables used in Libyan cuisine reflect local agriculture, making them fresh and readily available. According to the results outlined above, eating these raw vegetables can significantly increase the risk of contracting parasite infections. Communities that eat raw vegetables have been found to have high rates of intestinal parasites, particularly when those vegetables are grown on farms that use untreated human and animal fertilizers (26).

The current study discovered that seven different fresh vegetable varieties that are sold and regularly eaten by people in the Jardinah region were tainted with several parasitic stages. The health of people who eat raw vegetables from the area could be at risk. This study found that 45% of the vegetables had parasitic contamination in line with earlier reports from Amol, Iran, where contamination rates were 46.5% (30). This percentage is the highest among studies involving contamination rates in Mosul, Iraq, where they were 39%; (15) Nigeria, where they were 36%; (27) Benha, where they were 29.6%; (10) Alexandria, Egypt, where they were 31.7%; (28) and Brak Al-Shati, Libya, where they were 33%. (25) However, compared to Tripoli, where the contamination percentage reached 58% (29) and Benghazi, where it reached 90%, the contamination rates in this study were lower (11). There could be a number of reasons why this study differs from others. These could include differences in sample size, methods employed, climatic and environmental conditions, and geographic locations; Method of postharvest handling and socioeconomic standing (29, 31).

The study revealed a contamination rate of *G. lamblia* of 15%, and this finding is consistent with studies conducted in Ahvaz, Iran, where the contamination rate was 15.5%, (32) in Jiruft , Iran, where the rate was 14% (33), and in Benghazi, Libya, where the rate was 13.3%. (11)In contrary, lower contamination rates were recorded in Tripoli, Libya, 10%, (29) Brak Al-Shati, Libya, 3%, (25) and Amman, Jordan, 9%. (34) Capsicums were the most contaminated with *G. lamblia*, showing a rate of 54.5% (6/11), as shown in Table 3.

The parasitic stage that was second most common was *E. histolytica* in study 10%, comparable outcomes were discovered in Arba Minch, Ethiopia 8.4% (35) and Tabriz, Iran 8% (38). Nonetheless, several studies have revealed that high contamination rates were detected in Riyadh, Saudi Arabia, and Gaza Governorate, 35.5% and 37.5% respectively. (36, 37) Reduced rates were found in Manila, Philippines was 0.6% (39). Dill exhibited the highest contamination rate of *E. histolytica*, with a prevalence of 28.5% (4/14), as illustrated in Table 3.

The study found a parasitic contamination rate of 7% for *E. coli*, which is slightly lower than the 9.2% observed in Shahrekord, Iran, (40) but higher than the 2.5% detected in Manila, Philippines (39). A separate study reported the highest contamination rate in southwestern Saudi Arabia at 19.04% (41). Additionally, *E. vermicularis* eggs were found in 5% in this the study, comparable to rates of 5.1% in Khorramabad, Iran, (42) 4.9% in Benha, Egypt, (10) and 4.5% in Manila, Philippines (39) with higher rates observed in Zahedan, Iran 8.1% (43) and southwestern Saudi Arabia 6.3% (41). *Hymenolepis nana* eggs were found in a previous study in Benghazi, Libya, a rate of 13.3%,(11) which is higher than the current study's rate of 5% and also lower rates in Benha 2.8% (10) and Alexandri 2.6% (28). Cucumbers had the highest contamination rate of *E. coli*, with a prevalence of 30.7% (4/13) as presented in the Table 3.

Our study revealed the presence of *Cryptosporidium* oocysts 1.5 % Contrary to a previous study in which *Cryptosporidium* was the most prevalent parasitic contamination of green vegetable samples was 29.3% (28) vegetables in Nitro in Brazil, were reported 26% with *Cryptosporidium* infection (44).

In the present study, *A. lumbricoides* eggs were detected in 1.5 % of the examined samples. *A. lumbricoides* eggs were the least detected parasitic stages contaminating green vegetables 0.6% (10). The Iranian study proved the prevalence of *A. lumbricoides* in Iranian people varies between 1.5% and 53.2% (45). Parsley had the highest contamination rate of *E. vermicularis* 25% (3/12) and *A. lumbricoides* eggs 8.3% (1/12), In contrast, green onions exhibited a higher presence of *Cryptosporidium* spp Oocysts 13.3% (2/15) and *H. nana* 20% (3/15) eggs as indicated in Table 3.

Conclusion

The findings of this study clearly indicate that raw vegetables are often contaminated with parasites when consumed by humans. Consuming these contaminated vegetables, which harbor pathogenic parasites, without proper washing or preparation can be harmful to health. Prevention of contamination remains the most effective way to reduce the rates of foodborne parasite infections. It is essential for farmers, vendors, and the general public to receive comprehensive health education regarding the risks associated with eating contaminated vegetables and the importance of cleaning and disinfecting them before consumption. Therefore, it is strongly recommended to implement regulatory measures, including standards for irrigation water quality, preventing domestic and wild animals from accessing plant farms, and refraining from using untreated manure as fertilizer. Additionally, further research on parasite contamination in fruits and vegetables, as well as in the soils and water used for their cultivation, is advised, and these studies should be conducted in various regions of the country.

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