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Parasitic Contamination of raw vegetables sampled from different farm locations in Brack Al-Shati, Libya

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Abstract ***Background***: Fresh Vegetables provide important nutritional value for humans; however, consumption of raw vegetables can be a major route for transmission of food-borne parasitic diseases. Hence, this study aimed to investigate the parasitic contamination of commonly consumed local vegetables in Brack Al-Shati, Libya. ***Methods***: A total of 200 samples of fresh vegetables were randomly collected from vegetable farms in Brack Al-Shati, Libya. The vegetables included; green onion, lettuce, rocket, coriander, parsley, chard, mint, cucumber, carrot, tomato, and turnip. Samples were washed in saline, and the resulting washing solution was filtered and centrifuged to concentrate the parasitic stages. The sediments were examined by iodine and modified Ziehl-Neelsen stained smears. **Results**: The percentage of contamination was 33.0% (66/200), with predominance of nematode larvae (37.9 %), eggs of *Ascaris* (22.7%), cysts of *Entamoeba* spp. (18.2%), oocysts of *Cryptosporidium* spp. (16.7%), cysts of *Giardia lamblia* (3.0%) and eggs of *Hymenolepis nana* (1.5%). The highest number of contaminated samples was detected in rocket (70.1%) while the least number of contaminated samples was detected in green onion (6.7%). No parasite was detected in tomato samples.
Conclusion: The study highlighted the importance of vegetables as the potential source for transmission of intestinal parasites to humans. Vegetables contaminated with the pathogenic parasites may pose health risk to the consumers if consumed without proper cleaning and or cooking.
Keywords: Brack Al-Shati, Contamination, Intestinal parasites, Libya, Vegetables.

التلوث الطفيلي في عينات الخضروات المجمعة من المزارع الواقعة في مختلف مناطق براك–الشاطئ، ليبيا

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الملخص تعد الخضروات عنصر اساسي في الغذاء الصحي و المتوازن. الا ان استهلاك الخضروات و خصوصا تلك التي تؤكل نيئة يعد احد الطرق الهامة لانتقال الطفيليات المعوية و غيرها من الجراثيم الممرضة. اجريت هذه الدراسة للتحري عن التلوث بالطفيليات المعوية في بعض انواع الخضروات المحلية في منطقة براك– الشاطئ، ليبيا. **المواد والطرق**: تم جمع 200 عينة خضروات بشكل عشوائي من مزارع خضار بمدينة براك الشاطئ و ضواحيها. شملت الخضروات كل من: البصل الأخضر، الخس، الجرجير، الكسبرة، المعدنوس، النعناع، السلق، الخيار، الطماطم، الجزر و اللفت. فحصت العينات عن طريق غسلها بالمحلول الملحي و من ثم تركيز ماء الناتج من الغسيل باستخدام الطرد المركزي. تم فحص الراسب باستخدام صبغة اليود و صبغة زيل نيلسون المعدلة. **النتائج**: بلغت نسبة التلوث بالطفيليات في الخضروات المفحوصة 33.3% (200/66). شملت الطفيليات المعزولة من الخضروات: يرقات الديدان الخيطية Nematoda larvae (37.9%)، بيوض ديدان الاسكارس *Acaris* eggs (22.7%)، اكياس الانتاميبا *Entaomeba spp.* cysts (18.2%)، الاكياس البيضية للكربتوسبورديوم *Cryptosporidium* spp. oocysts (16.7%) اكياس الجارديا *Giardia lamblia* cysts (3.0 %) و بيوض الدودة الشريطية القزمة *Hymenolepis nana eggs* (1.5%). كانت نسبة التلوث بالطفيليات في الجرجير (70.1%) الاعلى من بين الخضروات المفحوصة، فيما سجل البصل الاخضر اقل نسبة تلوث بالطفيليات (6.7%). بينما لم يم عزل اي طفيليات من عينات الطماطم المفحوصة. **الخلاصة**: بينت هذه الدراسة أهمية الخضروات كمصدر مصدر لنقل الاصابة بالطفيليات المعوية. لذا فإن استهلاك الخضروات الملوثة قد يشكل خطر على صحة المستهلكين اذا لم يعتن بغسلها وتعقيمها قبل تناولها.
الكلمات المفتاحية: براك الشاطئ، تلوث، طفيليات معوية، ليبيا، خضروات.

1. Introduction

Fresh vegetables are key components of a healthy diet since they supply vitamins, minerals, and dietary fibers. They also contain phytochemicals which act as antioxidants, antibacterial, and have anti-viral and anti-carcinogenic properties [1, 2].

A lot of vegetables are consumed raw or lightly cooked to retain the natural taste and preserve heat labile nutrients. Consumption of raw vegetables can be an important route of transmission of several foodborne diseases because of their complex surface and porosity,

which unfortunately facilitate pathogen attachment and survival [3]. Vegetables can while growing in the fields or during harvesting, transportation, handling processes, distribution and marketing, even at home before consumption [4-6]. The extent of contamination depends on several factors that include, using contaminated water for irrigation, applying untreated manure as fertilizer, faecal contamination from wild and domestic animals, and human, post-harvest handling and hygienic conditions of preparation in food service or home settings [7, 8].

In recent years, there has been an increasing in number of reported cases of food-borne illnesses linked to consuming of fresh Vegetables [9, 10]. Consumption of vegetables generally eaten unwashed, uncooked and un-hygienically prepared, may lead to parasitic infections. Vegetables can transmit cysts and oocytsts of protozoa (e.g. *Giardia, Entamoeba, Toxoplasma*, *Cryptosporidium* and *Isospora*) as well as eggs and larva of helminthes (*Ascaris, Toxocara, Hymenolepis, Taenia, Fasciola, Enterobius, Trichuris, Trichostrogylus, Strongyloides,* and *hookworms*), that can cause diseases in humans [10-14].

To our knowledge, there is no previously published data about the contamination of fresh vegetables in Brack Al-Shati, Libya. Consumption of contaminated vegetables may pose a risk to the human health. Information about contamination of fresh vegetables is required to assist relevant authorities to take the proper actions to improve food safety and safeguard public health. Within this context, the present study aims at investigating the parasitic contamination of some vegetables from vegetable farms in Brack Al-Shati municipality.

2. Material and Methods

2.1. Study area

The study was conducted in Brack (27°32'45"N, 14°16'07"E), is a town in Wadi Al Shatii District in south-west Libya. It is the administrative centre of the district. The city is situated in the Fezzan valley, about 700 km of the capital city Tripoli. The area is characterized by desert climate, dry and hot weather and low rainfall. Agriculture is one of the main occupations of the people and underground wells are the main source of water.

2.2. Sample collection

This study was carried out during the period from January, 2018 to April, 2018. The study included 200 vegetable samples, comprised of eleven types of fresh vegetables: green onion (*Allium cepa*), lettuce (*Lactuca sativa*), rocket/arugula (*Eruca sativa*), coriander (*Coriandrum sativum*), parsley (*Petroselinum crispum*), chard (*Beta vulgaris*), mint (*Mentha piperita*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), carrot (*Daucus carota)*, and turnip (*Brassica rapa*). The samples were randomly collected from vegetable farms in Brack Al-Shati municipality. Each vegetable sample was placed in a separate nylon bag and labelled with a unique number and date of collection. The samples were transported to become contaminated with enteric bacterial, viral and parasitic pathogens

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2.3. Sample preparation and Examination

Approximately 200g of each vegetable was chopped into small pieces, washed with 500ml of sterile physiological saline solution (0.95% NaCl) and shaken for 15 min in order to separate the parasites from vegetables. Vegetable sample was removed and the remaining wash solution was left for 10 hours to sediment. The top layer was discarded and the remaining wash solution was filtered through sterile gauze to remove large debris and then centrifuged at 2000 rpm for 15minutes. The sediment was mixed and examined as follow:

- Direct smear: a drop of the sediment was applied on the center of a clean slide. A cover slip was placed gently to avoid air bubbles and over flooding. The preparation was examined under a light microscope using 10 × and 40× objectives.
- Iodine smear: a drop of the sediment was mixed with a drop of Lugol's Iodine solution and examined as in direct smear (three for each sample). Smears were used for detection of parasitic eggs, cysts and larva.
- staining smear: Modified Ziehl–Neelsen acid-fast stain was applied for staining of coccidian protozoa oocysts. Smears were used for detection coccidian protozoan oocysts including *Cryptosporidium* spp., *Isospora belli*, and *Cyclospora cayetanensis*.

3. Results

A total of 200 fresh vegetable samples were examined for the presence of parasite contamination. Intestinal parasites were detected in 33.0% (66/200) of the examined samples (Table 1).

Table 1 Parasitic contamination of fresh vegetables collected from farms in Brack Al-Shati, Libya

Vegetable	No. Examined	No. positive (%)
Rocket	17	12 (70.1)
Lettuce	17	9 (52.9)
Carrot	21	10 (47.5)
Parsley	45	16 (35.6)
Coriander	14	5 (35.7)
Mint	15	5 (33.3)
Cucumber	13	3 (23.1)
Chard	13	3 (23.1)
Turnip	17	2 (11.8)
Green onion	15	1 (6.7)
Tomato	13	0 (0)
Total	200	66 (33.3)

The parasites detected in the vegetable samples were nematode larvae (37.9 %), eggs of *Ascaris* spp. (22.7%) cysts of *Entamoeba* spp. (18.2%), oocysts of *Cryptosporidium* spp. (16.7%), cysts of *Giardia lamblia* (3.0%) and eggs of *Hymenolepis nana* (1.5%). The highest number of contaminated samples was detected in rocket (70.1%) followed by lettuce (52.9%), carrot (47.5%), parsley (35.6%), coriander, (35.7%), mint (33.3%), cucumber (23.1%), chard (23.1%) and turnip (11.8%) while the least number of contaminated samples was

detected in green onion (6.7%). No parasite was detected in tomato samples.

Presence of larvae/eggs of helminths, corresponded to 62.1% of the findings in the samples analyzed, which was higher than the presence of protozoan cysts/oocysts (37.8%). The species detected in the vegetable samples are summarized in Table 2 & Table 3. It can be noted that filariform and rhabditiform nematode larvae were the most frequent findings, followed by *Ascaris* spp. Eggs, *Entamoeba* spp. cysts and *Cryptosporidium* spp. oocysts.

Table 2 Intestinal parasites detected in fresh vegetables collected from farms in Brack Al-Shati, Libya

Parasite	No. positive (%)
Nematode larvae	25 (37.9)
Ascaris spp.	15 (22.7)
Entamoeba spp.	12 (18.2)
Cryptosporidium spp.	11 (16.7)
Giardia lamblia	2 (3.0)
Hymenolepis nana	1 (1.5)

Table 3 Distribution of intestinal parasites on each type of vegetable relation to the type of fresh vegetable samples

Vegetable	No. Examined	Nematode larvae	Ascaris spp.	Entamoeba spp.	Cryptosporidium spp.	Giardia lamblia	Hymenolepis nana
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Rocket	17	4 (23.5)	2 (11.8)	2 (11.8)	3 (17.6)	0 (0)	1 (5.9)
Lettuce	17	3 (17.6)	3 (17.6)	3 (17.6)	0 (0)	0 (0)	0 (0)
Carrot	21	7 (33.3)	1 (4.8)	0 (0)	0 (0)	2 (9.5)	0 (0)
Parsley	45	5 (11.1)	5 (11.1)	4 (8.9)	2 (4.4)	0 (0)	0 (0)
Coriander	14	1 (7.1)	0 (0)	1 (7.1)	3 (21.4)	0 (0)	0 (0)
Mint	15	2 (13.3)	2 (13.3)	1 (6.7)	0 (0)	0 (0)	0 (0)
Cucumber	13	0 (0)	0 (0)	1 (7.7)	2 (15.4)	0 (0)	0 (0)
Chard	13	1 (7.7)	1 (7.7)	0 (0)	1 (7.7)	0 (0)	0 (0)
Turnip	17	2 (11.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Green onion	15	0 (0)	1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)
Tomato	14	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	200	25 (37.9)	15 (22.7)	12 (18.2)	11 (16.7)	2 (3.0)	1 (1.5)

4. Discussion

Consumption of vegetable products is commonly viewed as a potential risk factor for infection with bacterial, viral, and parasitic foodborne diseases. Detection of intestinal parasites from vegetables is an indicative of the faecal contamination from human and/or animal origin and may be helpful in indicating the incidence of intestinal parasites among a given community [10]. In, Libya limited studies have been conducted to investigate the parasitic contamination on vegetables. Our study is the first that was carried out to determine the parasitic contamination of some of the commonly consumed fresh vegetables from vegetable farms in Brack Al-Shati municipality.

The present study has shown that 11 fresh vegetables routinely used for human consumption in Brack Al-Shati region were contaminated with several parasitic stages. The overall parasitic contamination rate was found to be 33.0%, which was consistent with previous reports from Egypt (31.7%) [13], and (29.6%), Syria (31.3%) [9] and Iran (32.6%) [15]. However, higher contamination rate (58.0%) was reported in in salad vegetables in Tripoli, Libya [16]. Higher contamination rates, ranging from 48.9% to 62.5%, were reported in Brazil [17], Iraq [18], Ethiopia [19] and Morocco [20]. In contrast, the rate of contamination was lower in some studies, such as 5.9% in Turkey [21], 16.2% in Saudi Arabia [10], and 13.5% in Sudan [14]. The differences between this study and others might be attributed to several factors. These may include the variations in geographical locations, climatic and environmental conditions, differences in the sample size, the techniques used; post-harvest handling method and socioeconomic status [16, 19].

In this study, helminth eggs and/or larvae were detected in 62.1% of the samples. Filariform and rhabditiform larvae of hookworm/ *Strongyloides* like parasite were the most prevalent parasite contaminating vegetable samples. Besides humans it is well known that some of these species can also parasitize several animals such as cattle, dogs and cats [22]. In addition, species such as *Strongyloides stercoralis* has a complex life cycle with a free-living stage in the environment that does not require a host for its proliferation, in addition to its parasitic mode of life [23]. Due to their morphological similarities and nature of the analyzed samples, it was not possible to identify the genus and species of larvae found. Nonetheless, presence of hookworms and/or *Strongyloides stercoralis* filariform larvae on the samples could be a source of infection, as larvae are capable of actively penetrating human skin or mucous membranes [17].

The second most prevalent contamination found in this study was eggs of *Ascaris* spp. (22.7%). Abougrain et al. [16] found that *Ascaris* spp. was the predominant intestinal parasite contaminating salad vegetables sold at wholesale and retail

markets in Tripoli-Libya. Furthermore, in studies performed in Saudi Arabia [24], Iran [25], and Nigeria [26] *Ascaris* spp. eggs were the most frequently isolated parasites in vegetables. This dominance might be associated with this parasite's ubiquitous distribution, and the resistant nature of the eggs that enables them to survive unfavourable conditions. The eggs can survive in the absence of oxygen, live for two years at 5–10 °C, and be unaffected by desiccation for two to three weeks [27].

The presence of nematode larvae and *Ascaris* eggs in vegetables can be due to the quality of water used for irrigation and the probable use of untreated manure. In a study done in Turkey, soil transmitted helminthes (mainly Ascaris eggs) were detected in 14% of fresh vegetables and in 84% of soil samples where vegetables were cultivated in 61% of irrigation water [28]. During the visits to the farms, we observed that majority of farmers used untreated animal manure as fertilizer for the crops, besides; the felids were kept without fences which may expose the crops to the contamination from wild and domestic animals. Using of animal manure as soil fertilizer is a well-known source of human pathogens that can survive there for a considerable time and therefore, contaminant structures may be transferred to the produce and remain there for a long time [17]. Moreover, transmission of pathogens can occur directly from animals, birds and insects. Many animals can act as reservoirs for human pathogens and if these animals come into contact with fresh produce, contamination can occur. However, further studies are needed to evaluate the level of contamination of irrigation water, fertilizer and soil in which vegetables are cultivated.

Cysts of *Entamoeba* spp. and *Giardia lamblia*, and oocysts of *Cryptosporidium* spp. were detected in 18.2%, 3.0% and 16.7% of the vegetable samples, respectively. This contamination is probably due to high viability of the cysts and oocysts in the environment and their capability of strongly adhering to surface of vegetables particularly the leafy green ones such as rocket, lettuce, parsley, spinach and leek. [22,29]. Although contamination of vegetables may occur in a variety of ways, it is most likely to happen before harvest, either by contaminated manure, sewage sludge, irrigation water, or directly from wild and domestic animals [13].

In the present study, the highest rate of contamination was detected in rocket (70.1%) and lettuce (52.9%) samples while the least number of contaminated samples was detected in green onion (6.7%). This could be due to the fact that the degree of contamination varies according to the shape and surface of vegetables. Green leafy vegetables as rocket, lettuce, coriander and parsley have uneven surfaces and makes parasitic eggs, cysts and oocysts attached to the surface of the vegetable more easily. In addition, the flexibility of the vegetables' leaves can facilitate contact with the soil during cultivation and, consequently, with helminth structures probably found in the soil [30] (Silva et al. 2005). On the other hand, vegetables like green onions, cucumber, and tomato have smaller surface areas and less contact with the ground, which can minimize the chances of contamination [7]. These results were in assent several previous studies in that rocket and/or lettuce had the highest rate of contamination [13, 14, 16, 31].

5. Conclusion

In conclusion, the results of this study clearly show that raw vegetables consumed by people are quite often contaminated with parasites. Vegetables contaminated with the pathogenic parasites may pose health risk to the consumers if consumed without proper cleaning and or cooking. Prevention of contamination remains the most effective way of reducing food borne parasitic infection. A comprehensive health education should be given to farmers, vendors and the general population on the health risks associated with consumption of contaminated vegetables and the importance of washing and disinfecting them before consumption. Hence, adopting control measures that cover guidelines of irrigation water quality, preventing domestic and wild animals from entering into the plant farms and avoiding using untreated manure as fertilizer is highly recommended. Furthermore, more studies on the parasitic contamination of fruits and vegetables, as well as water, and soil in which these produces are cultivated are highly recommended. These studies should also be conducted in different regions of the country.

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