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Detection of Entamoeba histolytica \E.dipar and Ascaris lumbricoides in fresh vegetables consumed collected randomly farms from Brack Al-shati, Libya

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ABSTRACT

Vegetables are important for human health because they contain proteins, fibers, minerals, and vitamins. However, Vegetables can be contaminated with enteric parasitic pathogens, this study aimed to investigate Parasitic contamination with E.histolytica \E.dipar and Ascaris lumbriciodes of fresh vegetable consumed after washing with tap water collected randomly from farms in Brak al-Shati, Libya. Methodes:The study focused on seven types of fresh vegetables: onion, carrot, turnip, lettuce, Rocket, parsley, and chard , 10 samples of each type checked without washing and after washing with tap water, Approximately 100 g ,washed with 250ml of (0.95% NaCl), shaken for 15 min, . Vegetable was removed and the remaining wash solution was left for 10 hours to sediment, the sediment examined follow: direct smear, Iodine smear,10x,40x.. Results: parasitic contamination withE. histolytica\E.dispar was detected 15.7% (11/70), Ascaris lumbriciodes 22.8% (16/70), the precentage of contamination is mixed between E. histolytica\E.dispar and Ascaris it was 18.5% (13/70), after washing with tap water was 20% (14/70)Ascaris lumbriciodes, 14.3% (10/70) E.histolytica\ E. dispar, 10% (7/70) mixed.

الكشف عن المتحولة الحالة للنسج و الصفر الخراطيني في الخضروات الطازجة المستهلكة المجمعة من مزارع عشوائية في منطقة براك الشاطيء، ليبيا

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الكلمات المفتاحية:
المتحولة الحالة للنسج
الصفر الخراطيني
تلوث
خضروات
ليبيا

الملخص

الخضروات مهمة لصحة الإنسان لاحتوائها على البروتينات والألياف والمعادن والفيتامينات. ومع ذلك ، يمكن أن يكون استهلاك الخضروات النيئة طريقاً رئيسياً في انتقال الأمراض الطفيلية ، هدفت هذه الدراسة إلى التحقق من التلوث الطفيلي بالمتحولة الحلة للنسج والصفر الخراطينيفي الخضروات الطازجة المستهلكة بعد غسلها بماء الصنبور،جمعت العينات من مزارع عشوائية من بلدية براك الشاطئ، الطرق: ركزت الدراسة على سبعة أنواع من الخضار الطازجة: البصل، الجزر، اللفت، الخس، الجرجير ، البقدونس، السلق، 10 عينات من كل نوع لكل نوع ، تم فحصها مباشرة بدون غسل وايضا بعد غسلها بالماء، 100 جرام،غمرت العينات في 250 مل (0.95٪ كلوريد الصوديوم)لإزالة الطفيلياتى الموجودة في الخضروات ،وضعت في الرجاج، تمت إزالة الخضار بالترشيح بواسطة شاش معقم، ترك محلول الغسل المتبقي لمدة 10 ساعات لغرض الترسيب، تم فحص الراسب عن طريق المسحة المباشرة، مسحة اليود، x.10و x40.

النتائج: نسبة التلوث كانت المتحولة الحالة للنسج15.7٪(70/11)، 22.8٪ (70/16الصفر الخراطيني، 18.5٪ (70/13)نسبة التلوث مختلطة بين المتحولة الحالة للنسج والصفر الخراطيني، بينما كانت نسبة التلوث بعد غسل العينات بالماء 20٪ (70/14) الصفر الخراطييني، 14.3٪المتحولةالحالة للنسج(70/10)، 10٪((70/7عينات مصابة بالمتحولة الحالة للنسج والصفر الخراطيني معا.

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Introduction:

Vegetables are important for human health because they contain proteins, fibres, minerals, and vitamins. However, they also play a role in the transmission ofprotozoan cysts andhelminthes eggs [1], Vegetables require a moist environment to grow, and these conditions favour the growth of endoparasites with unusual usage patterns [6], This contamination could be caused by a variety of factors, including the use of untreated wastewater or contact with sewage and raw dung used as fertilizer [2].

Vegetables can be contaminated with enteric parasitic pathogens throughout the planting process up to and including consumption [3], The level of contamination depends on several factors including, but not limited to, the use of contaminated water for irrigation, the use of untreated compost, or improper mixing as fertilizer., fecal contamination., of pets and humans, post-harvest handling and hygienic preparation conditions in the catering or home / environment [4]; [5]. Fresh vegetables can be a transmission medium or protozoal cysts, helminths, eggs and larvae ([6, 7, 8]. This can occur through occupational exposure or through consumption of vegetables contaminated with human or animal feces without adequate washing and disinfection[8],

Intestinal parasitic infections have a considerable distribution throughout the globe with the highest-burden in growing international locations wherein terrible non-public hygiene, environmental sanitation, socio-economic, demographic, and health-associated behaviours have documented to persuade their transmission [9, 10]. The maximum acquainted manner of an unfoldof the intestinal parasitic infections is ngestion of infected meals and water, but they will additionally unfold from human to human through fecal-oral contact[11],

Several previous studies have emphasized the medical importance of these infections, for example, reported that parasitic infections are associated with symptoms such as diarrhea, dysentery, weight loss, malnutrition, anemia, abdominal pain, and other gastrointestinal manifestations[12].

Furthermore, chronic parasitism impairs children's physical and cognitive development [13], According to the Global Burden of Disease Study (GBDS), amoebiasis was responsible for more than 55,000 deaths and 2.2 million disability adjusted life years (DALYs) in 2010, while cryptosporidiosis was responsible for more than 99,000 deaths and 8.3 million 14, 15], The majority of these deaths and disability-adjusted life years occurred in developing countries [16].

The most common infectious agents of humans are these helminthic infections. over 1 / 4 of the world population, which means around a pair of billion individuals are stricken by the helminthes parasite, and it's one in every of the key burdens of developing countries, particularly in children. [17, 18], The soil-transmitted helminthes (STHs), enter into the form from contaminated soil that contains eggs of roundworm, worm genus lumbricoides it's the foremost common of human helminthes and is distributed worldwide [19].

1. Material and methods:

2.1 Study area.

The study was conducted in the municipality of BrackAlShati (27o00'41 "N, 14o56" 27 "E), which is located in the region of Wadi Al-Shati in southwest Libya. BrackAlShati is one towns located in the Fezzan Valley, about 700 km from the capital, Tripoli.The region is characterized by its desert and arid climate, and its climate is predominantly hot.This region is well known for the agricultural practices in which various products food is planted. Underground wells are the main source of water that supplies both farms and residents.

2.2 Sample collection.

This study was carried out during the period from May 2021 to August 2021. The study focused on seven types of fresh vegetables: (onion (Allium cepa), carrot (Daucuscarota), turnip (Brassica rapa), lettuce (Lactuca sativa),Rocket (Eruca sativa), parsley (PetroselinuzZmcrispum)and wiss chard (Beta vulgaris), 10 samples of each type were collected. Samples were collected at random farms in the municipality of BrackAl-Shati, Libya. Each vegetable sample is placed in a nylon bag and labeled with a unique number and date of collection.The samples were transported to the laboratory of the Department of Medical Laboratory Sciences, Faculty of Engineering and Technology, Brack.

1.3 Procedure for Sample Preparation.

vegetable sample was placed in a separate nylon bag and labeled with a unique number and date of collection,each sample checked without washing and after washing with tap water, Approximately 100 g of each vegetable was chopped into small pieces, washed with 250ml 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:

2.3.1 Calibration of Microscope.

2.3.2 Direct smear: a drop (10µL)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.

2.3.3 Iodine smear: a drop(10µL) 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 parasites, cystsof *E.histolytica**E.dispar*, eggs of *Ascarislumbiciodes*, The preparation was examined under a light microscope using 10 × and 40× objectives.

2. Results:

The overall percentage parasitic contamination was detected in the samples 57.14%(40/70) with *E. histolytica* and *Ascarislumriciodes. E. Histolytic*(11/70) 15.7%, while *Ascarislumbriciodes* 22.8% (16/70), and the results showed mixed of both parasites were 18.5% (13/70), while the non-contamination rate in the samples was 42.8% (30/70).

The percentage of contamination after washing with water was 20% (14/70) with *Ascaris lumbriciodes*, 14.3% (10/70) *E.histolytica*\ *E. dispar*, 10% (7/70) the percentage of contamination mixed between the two parasites,and the results showed no contamination 55.7% (39\70).

Table1: overall presence of *E.histolytica/E. dispar*and *Ascarislumbricoides* in a selected vegetables

Parasite target	Withoutwashing (direct)		Washing with tap water	
	No. of samples Contaminated	% samples Contaminated (CI)	No. of samples Contaminated	% samples Contaminated (CI)
E.histolytica/E.dispar	11	15.7%	10	14.3%
Ascarislumbricoides	16	22.8%	14	20%
E.histolytica \E.dispar and Ascaris lumbricoides	13	18.5%	7	10%
Not seen	30	42.8	39	55.7%
Total	70	100%	70	100%

Table 2: Number of cysts and eggs in 40 field/10microliter sa. Preparation (*E.histolytica /E.dispar* and *Ascarislumbriciodes*)

sample types	No. of sample examined	E.histolytica\ E.dispar Without washing	E.histolytica\ E.dispar Washing with tap water	Ascarislumbreciodes Without washing	Ascarislumbreciodes Washing with tap water
		No. of eggs/40microscopic field/10microlitersa. preparation	No. of eggs/40 microscopic field/10microliter sa. Preparation	No. of eggs/40 microscopic field/10microliter sa. Preparation	No. of eggs/40.microscopic field/10microliter sa. Preparation
Onion (Allium cepa)	10	9	3	16	6
Carrot (Daucuscarota),	10	33	16	16	10
Turnip (Brassica rapa),	10	21	13	39	12
Lettuce (Lactuca sativa	10	30	11	32	11
Rocket (Eruca sativa),	10	5	1	24	8
Parsley (PetroselinuzZmcrispum)	10	6	2	66	16
Swiss chard (Beta vulgaris),	10	9	5	24	10

The table shows number of eggs *Ascaris lumbriciodes* and Cysts of *E.histolytica\E. dispar* in each type of vegetables that were examined directly as well as after washing with water, for each type 10 samples. Cysts of *E.histolytica\E. dispa* in Carrot and lettuce (30, 21/10)respectively, Green onion and chard were (9/10),Parsley and Rocket were (6,5/10) ,Cysts of *E.histolytica\ E.dipar* was less after washing samples with tap water, 16/10 for carrot s, Turnip and lettuce(13.11/10), the lowest number of infectious cysts in Rocket samples (1/10), the mineral samples were the most contaminated with eggs of *Ascaris lumbriciodes* 66/10,turnip and lettuce(39, 32/10), Rocket and chard (24 /10), the lowest rate of eggs *Ascarislumbriciodes* in onions and carrot, The number of Ascaris eggs was few after washing with tap water, parsley (16/10) turnip and lettuce (12,11/10), (10/10) carrot and chard species respectively, Rocket and green onion (8,6/10)**.**

Discussion:

Detection of intestinal parasites from plants indicates fecal contamination of human and / or animal origin which uses as fertilizer for planting, and be useful to indicate the presence of intestinal parasites in a particular community[20], Limited studies were conducted to investigate the vegetables parasitic contamination in Libya, and our study is the first to measure parasite contaminants of *Ascaris lumbriciode* and *E.histolytica\E.dispar* in fresh vegetables taken from random farms in the Brak al-Shati, Libya.

The study showed that the parasite contamination in general in the targeted vegetable samples were 57.1% and in agreement with the previous study mentioned in Tripoli, Libya, where the percentage of contamination was (58.0%) [6].and contradicted with study in Misurata was78%[21].higher than in the present study. It also agrees with the studies reported in Brazil [22].Iraq (Mahdi2013), Ethiopia [11], and Morocco.[23].Its percentage ranges between 48.9% to 62%. In the present study percentage contaminations in selected vegetables by *Ascarislumbriciodes* and *E. histolytica\E.dispar*were 23% and 16 % respectively but study by [21].percentage contaminations in selected vegetables by *Ascarislumbriciodes* and *E.histolytica\E.dispar*were 5% and 20% respectively.

Cysts of *E. histolytica\E.dispar* were detected in 15.7% during direct examination without washing, 14.3% after ter washing with tap water, The most contaminated vegetables were carrots and lettuce, and the fewest samples were watercress, This contamination is likely due to their ability to particularly adhere to the surface of vegetables [24]. Although contamination of vegetables can occur in a variety of ways, it is very likely to occur pre-harvest, at from contaminated manure,directly from wild animals and domestic animals [27].As a percentage of the herds intended for this breeding, 90% of them are open, or sewage sludge, irrigation water.

Through this study we have noticed that the majority of farmers use untreated organic manure as fertilizer for crops, besides; All farms were open without fences, which could expose crops to contamination from wild and domestic animals. The use of animal manure as soil fertilizer is a known source of human pathogens that can live there for a long time, thus, contaminated structures may transfer to the product and remain there for a long time [22].Moreover, transmission of pathogens can occur directly from animals to animals. Agricultural crops can work, as many animals are considered reservoirs for human pathogens, which causes pollution, and it is possible that contamination occurs with *E.histolytica /E.dispar* cysts and *Ascaris lumbricoides* eggs through irrigation water as well as soil, and accordingly; there is a need for more studies to assess the level of Contamination of irrigation water, fertilizers and the soil in which vegetables are grown, which are considered as a source of parasitic infestation.

3. Conclusion

In conclusion, the results of this study clearly showed that the raw vegetables that people consume are often contaminated with parasites. Vegetables contaminated with the pathogenic *Entamoeba histolytica* and *Ascaris lumbricoides* may pose health risks if consumed without proper cleaning and/or cooking. The present study was found that the vegetables that were targeted remain contaminated even after washing them with tap water, pollution prevention is still the most effective way to reduce food borne parasitic infection. Alternative methods should be found to eliminate and reduce the spread of *Entamoeba histolytica* and *Ascaris lumbricoides*in the vegetables that are being consumed Comprehensive health education should be given to farmers, vendors and the general population on the health risks associated with consuming contaminated vegetables and the importance of their washing and before consumption. Adoption of control measures covering irrigation water quality guidelines, Prevent domestic and wild animals from entering vegetable farms and avoid using untreated manure as high recommended fertilizer. Avoid using organic manure, instruct farmers on how to handle vegetables during harvest in order to prevent contamination, Do not use human faeces to fertilize vegetables, moreover, more Studies on the parasitic contamination of cultivated vegetables and fruits as well as the water and soil in which they are produced are highly recommended. Conducting studies on the use of alternative methods of washing with water to get rid of parasites that may cause contamination of vegetables, these studies should also be conducted in different regions of the country**.**

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