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Effectiveness of Common Washing Methods in Eliminating *Ascaris lumbricoides* Contamination from Fresh Vegetables Collected from Farms in Brack Alshatti

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ABSTRACT

Fresh vegetables provide important nutritional value for humans; however, they can become contaminated with enteric parasitic pathogens at various stages, from cultivation to consumption. Therefore, this study aimed to investigate the contamination of *Ascaris lumbricoides* in fresh vegetables collected from farms in Brack Al-Shatti, Libya, and to determine the most effective method for removing *Ascaris lumbricoides* egg contamination from vegetables. A total of 70 fresh vegetable samples were randomly collected from vegetable farms in Brack Alshatti. The vegetables included green onion, lettuce, rocket, parsley, chard, carrot, and turnip. Different washing methods were applied, including 5% vinegar, 10% salt, and 20% lemon solutions, in addition to washing with tap water. Microscopic examination for the presence of *A. lumbricoides* eggs was performed using a direct wet mount technique. Overall contamination with *A. lumbricoides* was detected in 22.9% (16/70) of the vegetable samples. After washing with tap water, parasitic contamination was detected in 20.0% (14/70) of the samples. Washing with 10% salt reduced the contamination rate to 11.0% (8/70), while washing with 20% lemon reduced it to 4.3% (3/70). The lowest contamination rate was observed after washing with 5% vinegar, at 2.9% (2/70). The results showed persistent contamination with *A. lumbricoides* in turnip and lettuce when all washing methods were applied. After washing with 10% salt, no contamination was detected in green onion. Furthermore, no contamination was observed in green onion, carrot, rocket, parsley, or chard after washing with 5% vinegar or 20% lemon. These findings highlight vinegar as a superior washing agent, effectively eliminating parasitic contamination while offering a safe and broadly effective solution.

دراسة فعالية طرق الغسيل الشائعة في القضاء على التلوث بدودة الصفر الخراطيني في الخضروات الطازجة المجمعة من مزارع في منطقة براك الشاطئ

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الكلمات المفتاحية

طفيل الصفر الخراطيني(الاسكارس)
الخضروات الطازجة
الإزالة
الفعالية
عوامل الغسل

الملخص

توفّر الخضروات الطازجة قيمة غذائية مهمة للإنسان، إلا أنّها قد تتعرض للتلوث بالطفيليات المعوية خلال مختلف مراحل الإنتاج، بدءًا من الزراعة وحتى الاستهلاك. هدفت هذه الدراسة إلى التحري عن تلوث الخضروات الطازجة بطفيل دودة الصفر الخراطيني في المزارع الواقعة بمنطقة براك الشاطئ – ليبيا، وتحديد أفضل طريقة لمعالجة الخضروات للحد من التلوث الطفيلي. تم جمع 70 عينة من الخضروات الطازجة بشكل عشوائي من مزارع الخضروات في منطقة براك الشاطئ، وشملت العينات: البصل الأخضر، والخس، والجرجير، والبقدونس، والسلق، والجزر، واللفت. واستُخدمت عدة طرق لغسل الخضروات، شملت الغسل بالخل بتركيز 5%، وبالمحلول الملحي بتركيز 10%، وبعصير الليمون بتركيز 20%، إضافةً إلى الغسل بماء الصنبور. وتم فحص العينات مجهريًا للكشف عن وجود بيوض دودة الصفر الخراطيني باستخدام طريقة التحضيرات الرطبة المباشرة. أظهرت النتائج أن نسبة التلوث الإجمالية بطفيل دودة الصفر الخراطيني بلغت 22.9% (70/16). وبلغت نسبة التلوث 20.0% (70/14) بعد الغسل بماء الصنبور، بينما انخفضت إلى 11.0% (70/8) بعد الغسل بالمحلول الملحي بتركيز 10%. كما بلغت نسبة التلوث 4.3% (70/3) بعد الغسل بعصير الليمون بتركيز 20%، في حين انخفضت إلى 2.9% (70/2) بعد الغسل بالخل بتركيز 5%. كما أظهرت النتائج استمرار وجود تلوث بطفيل دودة الصفر الخراطيني في عينات اللفت والخس عند استخدام جميع طرق الغسل. ولم يُلاحظ أي تلوث في عينات البصل الأخضر بعد الغسل بالمحلول الملحي بتركيز 10%. كذلك لم يُكشف عن أي تلوث في عينات البصل الأخضر، والجزر، والجرجير، والبقدونس، والسلق بعد الغسل بالخل بتركيز 5% أو بعصير الليمون بتركيز 20%. وتشير هذه النتائج إلى أن الخل يُعدّ الوسيلة الأكثر فاعلية لغسل الخضروات وإزالة الطفيليات، لما يوفره من خيار آمن وفعّال على نطاق واسع.

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Introduction

Although there have been many research focused on protozoan parasites, food-related pathogens such as *Ascaris lumbricoides* have not been investigated [1, 2]. Vegetables are an essential component of a balanced diet, as they contain proteins, fibers, minerals, and vitamins [3]. However, fresh vegetables can serve as a transmission medium for protozoan cysts, helminths, eggs, and larvae. The level of parasitic contamination of vegetables depends on several factors, including the use of contaminated water for irrigation, the application of untreated manure as fertilizer, fecal contamination from wild and domestic animals and humans, post-harvest handling practices, and hygienic conditions during preparation in food service establishments or home settings [3, 4].

The consumption of fresh, raw, and unwashed vegetables may increase the risk of infections and illnesses resulting from contamination with intestinal parasites, such as *Ascaris lumbricoides* [5, 6].

A. lumbricoides is a large soil-transmitted nematode that causes ascariasis. Transmission occurs through the ingestion of embryonated eggs from contaminated soil, food, and/or water. *Ascaris* eggs are highly resistant and have the potential to survive for extended periods, particularly under warm and moist environmental conditions [7]. Approximately one billion people worldwide are infected with *A. lumbricoides*, and more than 60,000 deaths are reported annually as a result of the disease. Ascariasis predominantly affects tropical and subtropical regions and is frequently documented in Sub-Saharan Africa, Latin America, China, and East Asia [8, 9]. Infections caused by *A. lumbricoides* are classified among the world's neglected tropical diseases.

According to the Institute for Health Metrics and Evaluation (IHME), the global burden of ascariasis in 2019 for both sexes combined was estimated at a prevalence of 446,000 cases (95% CI: 394,000–511,000), 754,000 disability-adjusted life years (DALYs) (95% CI: 479,000–1,140,000), and a total of 2,090 deaths (95% CI: 1,640–2,640) (Institute for Health Metrics and Evaluation, University of [4]. Ascariasis ranked 195th in 2010 and 203rd in 2019 among the most common causes of death worldwide, and 215th in 2010 and 235th in 2019 in terms of DALYs. The percentage change between 2010 and 2019 was −17.4% for prevalence, −26.3% for DALYs, and −42.9% for deaths [10].

Ensuring food safety and mitigating the risk of parasitic contamination require the implementation of rigorous preventive measures. Raw vegetables should be thoroughly washed under running potable water, with consideration given to the use of approved disinfectants when appropriate. Vegetable decontamination may be achieved through physical, chemical, or biological washing approaches, each demonstrating varying levels of effectiveness in removing parasitic contaminants [11].

Consistent adherence to proper washing practices is essential for preventing outbreaks of intestinal parasitic infections [12]. Nevertheless, only a limited number of studies have compared the effectiveness of different washing methods in eliminating parasitic contamination from vegetables [13, 14, 15]. This ongoing public health concern highlights the urgent need for cost-effective, practical, and accessible household decontamination techniques that do not require specialized equipment or expensive resources. Therefore, the present study aimed to investigate contamination with *A. lumbricoides* in fresh vegetables and to evaluate the effectiveness of different washing methods in eliminating *A. lumbricoides* egg contamination.

Materials and Methods

Study Area

The study was conducted in the municipality of Brack Al-Shatti (27°00'41" N, 14°56'27" E), located in the Wadi Al-Shati region in southwest Libya. Brack Al-Shatti is a town situated in the Fezzan Valley. The region is characterized by a desert and arid climate, predominantly hot. It is well known for its agricultural practices, where various crops are cultivated. Underground wells serve as the main source of water supplying both farms and residents.

Sample Collection

This study was carried out during the period from March to August 2023. It focused on seven types of fresh vegetables: carrot, green onion, turnip, lettuce, rocket, parsley, and chard. Ten samples of each type were collected randomly from farms in the municipality of Brack Al-Shatti, Libya. Each vegetable sample was placed in a nylon bag and labeled with a unique number and the date of collection. The samples were then transported to the laboratory of the Department of Medical Laboratory Sciences, Faculty of Medical Technology, University of Wadi Al-Shati, Brack.

Sample Preparation and Microscopic Examination: The vegetable samples in this study were processed using the following methods:

1. Preparation of samples without washing: Approximately 100 g of each vegetable was chopped into small pieces and suspended without washing in 250 mL of sterile physiological saline solution (0.9% NaCl).
2. Preparation of samples with tap water: 100 g of each sample was washed with tap water, then chopped into small pieces and suspended in 250 mL of sterile physiological saline solution (0.9% NaCl).
3. Preparation of samples with 5% vinegar: 100 g of each sample was washed with 5% vinegar, then chopped into small pieces and suspended in 250 mL of sterile physiological saline solution (0.9% NaCl).
4. Preparation of samples with 10% salt: 100 g of the sample was washed with 10% salt, then the vegetable samples were chopped into small pieces and suspended in 250 mL of sterile physiological saline solution (0.9% NaCl).
5. Preparation of samples with 20% citrus lemon: 100 g of the sample was washed with 20% citrus lemon, then the vegetable samples were chopped into small pieces and suspended in 250 mL of sterile physiological saline solution (0.9% NaCl).

After subjecting the samples to the aforementioned preparation methods, the 250 mL suspension (0.9% NaCl with 100 g of sample) was shaken for 15 minutes to separate the parasites from the vegetables. The vegetable samples were then removed, and the remaining wash solution was left to sediment for 10 hours. After that, the top layer was discarded, and the remaining saline solution was filtered through sterile gauze to remove large debris and then centrifuged at 2000 rpm for 15 minutes. Finally, the sediment was mixed and subjected to microscopic examination [6, 13, 16] as follows:

1. **Direct smear:** A drop (20 µL) of the sediment was applied to the center of a clean slide. A cover slip was placed gently to avoid air bubbles and overflooding. The preparation was examined under a light microscope using 10× and 40× objectives.

2. **Iodine smear:** A drop (20 µL) of the sediment was mixed with a drop of Lugol's iodine solution and examined as in the direct smear.
3. **Egg counting:** The number of *A. lumbricoides* eggs was microscopically counted under a 40× objective.

This study represents a novel approach to investigating the efficacy of washing vegetables with salt, vinegar, and citrus lemon.

Statistical Analysis

Data were initially entered into Microsoft Excel for arrangement and expressed as percentages. SPSS (version 16.0) and Minitab (version 18) software were used for statistical analysis. Chi-square test and ANOVA were carried out to assess significant differences among the washed groups. A p-value of less than 0.05 was considered statistically significant [6, 13, 16].

Results:

In the present study, *A. lumbricoides* eggs were morphologically characterized as previously described by [13, 16]. *A. lumbricoides* eggs are usually classified into two forms: unfertilized and fertilized eggs (Figure 1). Unfertilized eggs have a corticated outer surface and are enclosed with an amorphous mass of protoplasm. Their size ranges from 85 to 95 µm by 38 to 45 µm. Fertilized eggs have similar measurements (40 to 75 µm by 30 to 50 µm) to those of unfertilized eggs. The outer morphology appeared either decorticated or corticated, and their shell seemed to be thicker than that of unfertilized eggs [17].

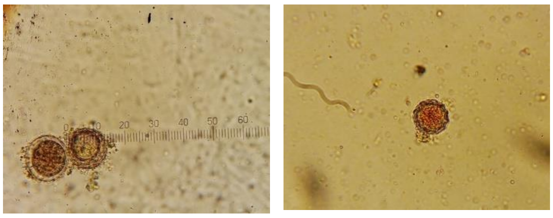

Figure 1: Eggs of *A. Lumbricoides* in iodine smear, 40x

The overall contamination with *A. lumbricoides* was 22.9% (16/70) of the vegetable samples. After washing the vegetables with tap water, parasitic contamination was detected in 20.0% (14/70) of samples; after washing with 10% salt, the contamination rate was 11.0% (8/70); while after washing with 20% citrus lemon, the contamination rate was 4.3% (3/70); and after washing with 5% vinegar, it was 2.9% (2/70). There were no statistically significant differences between the direct examination (without washing) and examination after washing with tap water or with 10% salt (p>0.05), while there were statistically significant differences between the direct examination and processing methods with 5% vinegar and 20% citrus lemon (p<0.05) (Table 1).

Table 1: Overall Assessment of Effective Washing Methods for Removal of *A. lumbricoides*

Washing Method	No. of Contaminated vegetable samples	(%) of Contaminated samples
Washing Method	16	22.9%
Without washing	14	20.0 %
Tap water	8	11.4%
10% Salt	2	2.9%
5% Vinegar	3	4.3%

The results showed the presence of contamination with *A. lumbricoides* eggs in turnip and lettuce when using all washing methods. After washing with 10% salt, no contamination was found in green onion. No contamination was detected in green onion, carrot, rocket, parsley, and chard after washing with 5% vinegar and 20% citrus lemon. There were no statistically significant differences between the washing methods (P>0.05) (Table 2).

Discussion

Fresh vegetables provide important nutritional value for human health; however, consumption of fresh vegetables without proper washing is a significant transmission route for parasitic protozoans and helminths [6,18,19]. *A. lumbricoides* is widespread in many areas of Libya due to inappropriate and unsanitary conditions that facilitate stool contamination of water, soil, and agricultural crops [20]. In this study, parasite contamination with *A. lumbricoides* eggs was 22.9% (16/70). This may be attributed to the eggs' ability to resist environmental conditions, as *A. lumbricoides* eggs can survive for up to two years and are not affected by drought for two to three weeks.

The highest number of eggs was detected in parsley samples (66/HPF), while the lowest was observed in green onion and carrot samples (16/HPF). Parasitic contamination of fresh vegetables in this study could be due to the type of irrigation water used and the type of fertilizer, as 90% of sampled farms used untreated animal manure. Most farms were unfenced, exposing crops to contamination from wild and domestic animals. This study is the first to use alternative washing and processing methods to remove parasitic contamination from vegetables.

The contamination rates of *A. lumbricoides* were 20%, 11.4%, 2.9%, and 4.3% according to the processing methods: without washing, washing with tap water, 10% salt, 5% vinegar, and 20% citrus lemon, respectively. Washing

Table 2: Number of *A.lumbricodies* eggs (HPF 40×) contaminating vegetable samples using different washing meth

Sample Type	No. of sample examined	Without washing	Washing with tap water	Washing with 10% salt	Washing with 5% vinegar	Washing with 20% citrus lemon
		No. of eggs per (HPFx40/20µL)	No. of eggs per (HPFx40)/20µL)	No. of eggs per (HPFx40/20µL)	No. of eggs per (HPFx40/20µL)	No. of eggs per (HPFx40/20µL)
Green onion	10	16	6	0	0	0
Carrot	10	16	10	1	0	0
Turnip	10	39	12	13	2	6
Lettuce	10	32	11	5	3	1
Rocket	10	24	8	3	0	0
Parsley	10	66	16	3	0	0
Chard	10	24	10	3	0	0
Total	70	217	73	28	5	7

vegetables with tap water showed that samples were still contaminated with *A. lumbricoides*, although the number of eggs was nearly halved. These results indicate the high resistance of *A. lumbricoides* eggs and their ability to adhere to vegetable surfaces. Similar results were reported by [20].

After washing with 10% salt, the highest number of *A. lumbricoides* eggs was detected in turnip (13/HPF). The rough surface of turnip makes egg removal more challenging compared to the smooth surface of lettuce. Although salt solution can aid in removal, surface texture affects the ease of egg dislodgement. Washing with citrus lemon solution reduced Ascaris egg retention on turnip to 6/39 and lettuce to 1/32, indicating greater efficacy on lettuce, followed by rocket, parsley, and chard (3/HPF), and the lowest number in carrot samples (1/HPF). Eggs were detected only in lettuce and turnip samples after washing with 5% vinegar and 20% citrus lemon solutions, demonstrating that vinegar was more effective in removing parasites compared to other washing methods [20]. Similarly showed that vinegar not only removes parasites more effectively than conventional methods but also inactivates pathogens, providing a natural, safe, and broadly effective solution.

Local farmers in Brack-Al-Shatti commonly use animal dung as organic fertilizer. While farmers claim that organic fertilizers produce higher yields than chemical fertilizers, they often lack awareness regarding parasite transmission via waste and animal/human feces. Farm visits revealed that many animals, including dogs, cats, cows, and sheep, roam freely in farms, contributing to contamination of crops and parasite transmission [21,22,23].

The contamination of certain vegetables with *A. lumbricoides* can be explained by their immersion in soil and surface roughness, as in carrots and turnips, which facilitates egg adhesion. Green onion samples were less contaminated due to smaller surface areas and minimal soil contact, although some eggs were detected due to the resistant nature of *A. lumbricoides* eggs. Parsley and lettuce were among the most contaminated vegetables due to their uneven surfaces and flexible leaves, which facilitate contact with soil or organic fertilizer during cultivation [6]. Irrigation water contamination and the presence of wild animals in unfenced or poorly fenced farms further contributed to parasitic contamination. Some farmers also graze livestock inside farms, increasing vulnerability of vegetables to parasitic contamination. [24].

Conclusion

This study reported a relatively high rate (22.9%) of vegetable contamination with *A. lumbricoides* eggs. Among vegetables, parsley was highly contaminated, while green onion and carrot showed lower contamination rates. Washing with 5% vinegar was significantly more effective in removing parasitic contamination compared to other washing methods, providing a natural, safe, effective, and affordable sanitary method. This study highlights vinegar’s role as a mechanical cleansing agent, establishing it as an accessible approach for ensuring household food safety.

Recommendations

1. Adoption of control measures covering irrigation water quality guidelines.
2. Preventing domestic and wild animals from entering vegetable farms.
3. Avoiding the use of untreated manure as fertilizer and instructing farmers on proper handling of vegetables during cultivation and harvest.
4. Further studies on parasitic contamination of cultivated vegetables, fruits, water, and soil are highly recommended.

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