

A Molecular Study of Parasitic Contamination of Fresh Leafy Green Vegetables Sold in Diwaniyah City, Iraq

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<p>Abstract: A healthy human diet must include vegetables because of their high nutritious content. However, when contaminated, they can transmit parasitic intestinal diseases. This study's objective was to evaluate the degree of parasite contamination in raw vegetables, especially since it is well known that these vegetables are present on the table of every Iraqi family in daily meals. A descriptive analytical research was conducted to ascertain the degree of <i>Giardia intestinalis</i> parasite contamination in vegetables sold in local marketplaces in Diwaniyah Governorate where 80 samples of five different types of vegetables was collected: <i>Lactuca stavia</i> (Lettuce), <i>Raphanus sativs</i> (radish), <i>Petroselinum crispum</i> (Parsley), <i>Allium porrum</i> (leek), <i>Nasturtium officinale</i> (Cress), purchased during the period from October 2024 to April 2025 than , it was brought to the laboratory for microscopic examination to determine the parasitic infection. The study's findings demonstrated that the highest contamination was (25%) in lettuce, (18.75%) in parsley, and (12.5%) in both radish and watercress, while the least contamination was leeks (6.25%). The study demonstrated the significance of vegetables as a possible human intestinal parasite source. Therefore, consumers ought to be informed of the need to wash them well before eating, in addition to the need for health education and dissemination of healthy practices among sellers and consumers.</p>	<p style="text-align: center;">Research Paper</p>
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INTRODUCTION

Vegetables are an essential part of the Mediterranean diet, providing vital nutrients and encouraging fullness [1]. They are a crucial component of a nutritious diet due to their vitamin and mineral content, and phytochemicals that are anti-inflammatory, phytoestrogens, and antioxidants [2]. Over the past ten years, individuals' dietary practices have shifted to include more veggies in their diets due to healthier lifestyle choices. The demand for fresh veggies has increased as a result [3]. Eating a range of veggies can help you control your weight, lower your risk of obesity, stroke, cardiovascular disease [4]. In order to gain vital nutrients and avoid chronic illnesses, the WHO also advises eating at least 400 grams of veggies per day [5]. Human diseases can contaminate vegetables at any stage of the farm-to-fork chain, including before and after harvest [6, 7]. The main sources of contamination at preharvest include soil, water, and manure (from humans or animals) [8].

However, these veggies could be significant carriers of enteric bacterial, viral, and parasite diseases if they are eaten raw without sufficient washing [3-10]. Consumption of contaminated fresh vegetables has been linked to food-borne parasite infections [11]. Generally speaking, fruits and vegetables are thought to be easy carriers of parasites, particularly when consumed fresh or without peeling [12]. In tropical and subtropical nations, where access to sanitary facilities and clean water is restricted and personal hygiene habits are inadequate, public health is seriously threatened by intestinal parasite infections [13, 14]. Raw leafy green vegetables are frequently consumed in Arab nations, particularly in Iraq. Previous research has demonstrated that eating fresh, unwashed vegetables has a major impact on the occurrence of clinically relevant parasites. Southern Thailand, Libya, Saudi Arabia, Ethiopia, Egypt, Brazil, Iran, and the Philippines are among the places where this has been noted [15,16-22]. Additionally, epidemiological

research revealed that the number of documented instances of food-borne infections brought on by eating raw fruits and vegetables has been rising, and that intestinal parasite infections continue to pose a significant risk to public health in endemic areas [23, 24].

There aren't many research on possible vegetable contamination in Iraq, and a 2013 study in the Al-Diwaniyah Governorate revealed that some examined samples of particular fruits and vegetables tested positive for parasite contamination [25]. In order to stop the spread of intestinal parasite infections, it is necessary to monitor the level of contamination in local settings. Therefore, the current study's objective was to ascertain the degree of parasite infection of leafy vegetables in particular local markets in the Iraqi governorate of Al-Diwaniyah.

MATERIALS AND METHODS

Sample Collection

In this investigation, 80 fresh leafy green vegetable samples were gathered from the local markets in Al-Diwaniyah city, [16], samples for every one of these veggies over the course of the (1\10\ 2024, to 1\4\2025) where samples were obtained randomly from the local marketplaces. Those vegetables included *Lactuca stavia* (Lettuce), *Raphanus sativs* (Radish), *Petroselinum crispum* (Parsley), *Allium porrum* (Leek), *Nasturtium officinale* (Cress), then the samples were then placed in individual plastic bags, labeled with their titles, and sent to the laboratory to search for protozoan *Giardia intestinalis* cyst stages.

Analysis of Samples

Following the washing of the veggies with a substantial volume of distilled water, the water was filtered through medical gauze to eliminate any sizable particles. The precipitate was then allowed to precipitate gradually where the refined water was poured, and five millimeters of the precipitate were taken and placed in test tubes and centrifuged for five

minutes at a speed of two thousand cycles per minute. After then, the purified liquid will be emptied, and a portion of the precipitate will be taken, placed on a slide, and inspected under a microscope to look for *Giardia intestinalis* protozoa cysts [26, 27].

PCR

The Genomic DNA extraction kit (Geneaid, Korea) was used to extract the DNA, and the kit's instructions were followed. A Nanodrop was used to estimate the quantity and quality of the DNA. A PCR method that targets the glutamate dehydrogenase (GDH) was used as a defining and verifying step to detect *Giardia sp*, the targeted portion had a length of 778 bp and the primers used were from (28), and they are F: ATCTTCGAGAGGATGCTTGAG and R: AGTACGCGACGCTGGGATACT. The Bioneer (South Korea) PCR mastermix kit was used, and 10 pmol of each primer was used in accordance with the kit's recommendations. The PCR conditions included an initial denaturation at 95°C for 3 minutes, 39 cycles of (denaturing at 95°C for 35 seconds, annealing at 54°C for 35 seconds, and extension at 72°C for 35 seconds) and a final extension at 72°C for 5 minutes. PCR results were conducted on an ethidium bromide-pretreated 1.5% agarose gel. A UV imager was used to visualize the product separation.

RESULTS

Microscopically

According to the current study's findings, It was discovered that 12 of the 80 vegetable samples were tainted with the *Giardia* parasite, resulting in an overall contamination rate of 15% (see table 1). The rate at which each vegetable contaminant spreads is also displayed in the table. Lettuce was the most infected vegetable species (25%) followed by parsley (18.75%), than radish and cress (12.5%), whereas the least contamination rate was in leek (6.25%), all these percentages of contamination of leafy green vegetables with the *Giardia* parasite show in Figure 2.



Fig. 1: Cysts of *Giardia*

Table 1: Giardia parasite prevalence in fresh vegetables vendors sell in Al-Diwaniyah city

Type of Vegetable	No. Examined	No. Positive (%)
Lettuce	16	4 (25%)
Radish	16	2 (12.5%)
Parsley	16	3 (18.75%)
Leek	16	1 (6.25%)
Cress	16	2 (12.5%)
Total	80	12 (15%)
Calculated P value	0.627 (NS)	

NS: P<0.05 indicates no significant difference

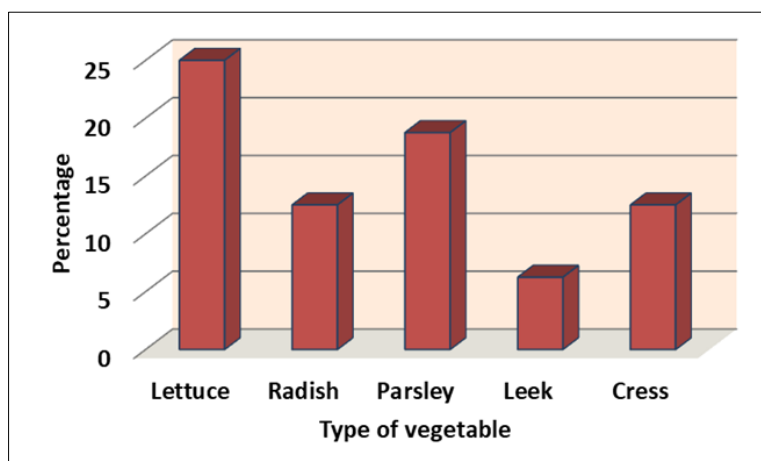


Fig. 2: Shows the percentages of contamination of leafy green vegetables with the Giardia parasite

PCR

The results of the investigated positive samples revealed that 100% of these samples contained

the *gdh* gene. The amplicons of size 778 bp displayed a distinct single distinctive band in Figure 3.

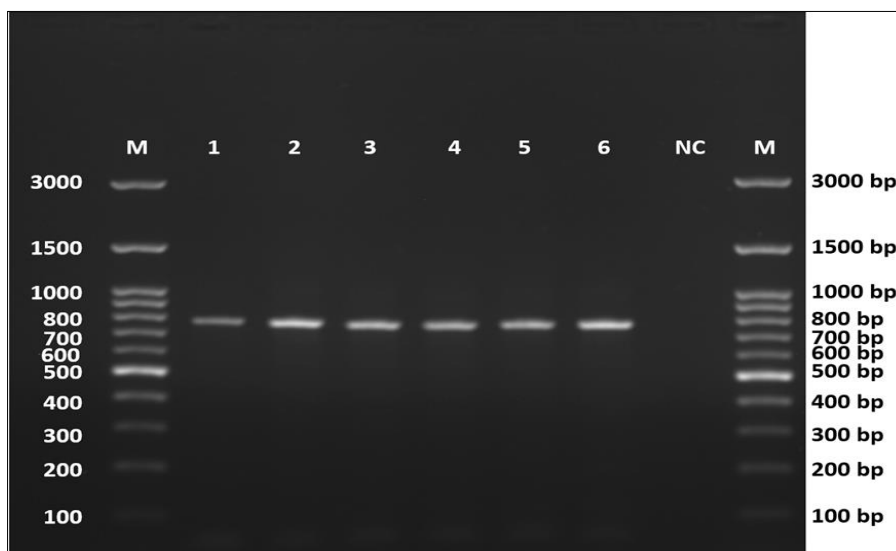


Fig. 3: An image of agarose gel electrophoresis displaying the PCR product of the GDH gene (size = 778 bp), with Lane (M) serving as the molecular weight marker

DISCUSSION

In addition to being a sign of animal or human fecal contamination, the identification of medically significant intestinal parasites in vegetables implies that vegetables may be a source of foodborne illness in

humans. Their presence in the vegetables is linked to both sanitary conditions and hygienic procedures that promote the spread of parasites as well as environmental circumstances that are conducive to their existence [29]. The results of Jimma City, Oromia, Ethiopia, which showed an overall prevalence of

15.2%, are consistent with the current study's overall parasite contamination rate of 15% [30], and approaching to what was recorded in Aden governorate, Yemen (13.9%) [31], and in Bahir Dar City, Northwest Ethiopia (10.2%) [32]. But compared to Saudi Arabia's Hail Region, this rate was higher (7.6%) [33]. The lengthy life times of the cyst form of *Giardia intestinalis* in wet environments and its resilience to numerous unfavorable conditions may be the cause of the high prevalence rate of *Giardia intestinalis* observed in our study [36]. However, the incidence of these parasites varies by region and is typically linked to unsanitary practices, inadequate access to clean water, and poor hygiene [37]. On the other hand, it is less than what was found in Damascus, Syria (38.6%) [34], and in a few rural parts of Egypt's Sharkya Governorate (32.3%) [35]. However, differences in geographic locations, meteorological and environmental circumstances, sample type and size studied, sampling methodologies, techniques for detecting intestinal parasites, and socioeconomic level could all account for discrepancies in contamination rates [38]. Of the five vegetable types included in the study, the greatest prevalence of *Giardia intestinalis* parasite was found in Lettuce 25%, consistent with [34], and [35] while Watercress was the most contaminated vegetable in [33], and parsley in [31]. Leek was the least contaminated vegetable (6.25%). These findings can be explained by the rough surfaces of lettuce leaves, which allow parasites to adhere to them and preserve their cysts when washed with water, in addition to the fact that lettuce contains a very short stem, which allows for greater contact with the soil and irrigation water. We used glutamate dehydrogenase gene to characterize the *Giardia* parasite via polymerase chain reaction (PCR) due to (GDH) gene is a dependable tool for molecular diagnostics since it has a distinct promoter, lacks typical eukaryotic features, and is a single-copy gene without introns as well as the numerous studies like [28-39].

CONCLUSION AND RECOMMENDATION

Studies on intestinal parasites in fresh vegetables, in Iraq are few compared to other studies in other countries therefore, this study emphasized the significance of raw vegetables as a possible human intestinal parasite transmission source. Given the high rate of contamination with various parasitic species, we call attention to monitoring the water used for irrigation and treating the organic fertilizer used for fertilization in a proper manner, in order to reduce contamination rates as much as possible and the customers should always follow the fundamental rule of food hygiene, which is to thoroughly wash vegetables before consuming them. We can recommend washing vegetables with white vinegar, as it contains acetic acid, which is an antimicrobial agent and has a bactericidal effect on many microorganisms. Also, Vegetable vendors and the general public should get thorough health education about the dangers of eating tainted

veggies. A way to keep an eye on how contaminated the vegetables sold at local markets are all the time should be established by the local public health sector.

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