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Research Article

Prevalence of Intestinal Protozoan Parasites in Fresh Leafy Vegetables Sold in Benghazi Markets, Libya: A Cross-Sectional Study with Food Safety Implications

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Abstract

Background: Leafy green vegetables are essential components of the Mediterranean diet but are highly susceptible to contamination with intestinal protozoan parasites due to their morphology, direct soil contact, irrigation water quality, and post-harvest handling. In Libya, particularly Benghazi, updated epidemiological data on vegetable-borne parasites remain scarce.

Objective: This study aimed to determine the prevalence and distribution of intestinal protozoan parasites in five commonly consumed leafy vegetables sold at Al-Funduq central market in Benghazi, Libya.

Methods: A total of 100 fresh samples (20 each of arugula, parsley, coriander, chard, and dill) were randomly collected. Parasitic stages were detected using standard sedimentation-concentration techniques (0.9% saline soaking for 24 h, centrifugation at 4000 rpm for 10 min) and light microscopy (×40) with iodine staining. Statistical analysis used Chi-square test (SPSS, $p \leq 0.05$).

Results: Overall contamination rate was 68%. Arugula showed the highest contamination (85%), followed by coriander (80%), parsley (75%), while chard and dill had 50% each. Entamoeba coli was the most prevalent (25 cases, 17%), then Entamoeba histolytica (20 cases, 13.6%), Giardia spp. (17 cases, 11.6%), and Isospora spp. (6 cases, 4.08%). Significant differences were found among parasite prevalence ($\chi^2 = 11.41$, $p<0.05$).

Conclusion: The high contamination rate (68%) exceeds global averages (~ 20%), indicating poor irrigation water quality and inadequate hygiene. Urgent interventions are required to improve water treatment, handling practices, and public awareness to reduce foodborne parasitic infections.

Keywords: Leafy vegetables, Intestinal protozoa, Entamoeba histolytica, Giardia, Benghazi, Food safety

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Introduction

Leafy green vegetables (*Eruca sativa*, *Petroselinum crispum*, *Coriandrum sativum*, *Beta vulgaris* subsp. *cicla*, *Anethum graveolens*) are cornerstones of the traditional Libyan diet. They are consumed fresh almost daily in salads such as *"salatata jarjeer"* (arugula salad), *"tabbouleh"* (parsley-based), and as garnishes for soups, stews, and rice dishes. Nutritionally, these vegetables are rich in vitamins (A, C, K, folate), minerals (iron, calcium, magnesium), dietary fiber, and bioactive antioxidants including flavonoids, carotenoids, and phenolic compounds. A large body of evidence, including the landmark review by Slavin and Lloyd **[1]**, has confirmed that regular consumption of fresh vegetables significantly reduces the risk of several non-communicable diseases: cardiovascular disease (by improving lipid profiles and reducing blood pressure), type 2 diabetes (via improved glycemic control and insulin sensitivity), certain cancers (colorectal, gastric, and breast), and all-cause mortality. Furthermore, the high fiber content promotes gastrointestinal health by enhancing bowel regularity, modulating the gut microbiome, and reducing inflammation.

Despite these well-established health benefits, the same morphological and agricultural features that make leafy greens nutritious also render them highly vulnerable to biological contamination, particularly by intestinal protozoan parasites. Unlike smooth-skinned fruits or root vegetables, leafy greens possess complex surface architectures: arugula has deeply lobed, rough, and creviced leaves; parsley has finely divided, curled surfaces that trap water and particulate matter; coriander has delicate, fern-like leaflets that grow close to the ground; chard has broad, fleshy leaves with prominent veins; and dill has highly dissected, thread-like leaves. These structural features create microhabitats where parasitic cysts and oocysts can attach, resist washing, and remain viable for extended periods **[2].**

The contamination pathway begins at the farm level. In many peri-urban areas surrounding Benghazi, including the agricultural plains of Al-Marj, Qaminis, and Suluq, farmers often rely on untreated or partially treated groundwater, and in some cases, untreated wastewater from open drainage channels, due to the high cost of treated water and intermittent municipal supply. This practice introduces fecal matter containing protozoan cysts directly onto edible plant surfaces via splash irrigation, flooding, or subsurface wicking. Additionally, the use of uncomposted or inadequately composted animal manure as organic fertilizer-common among smallholder farmers-adds another layer of risk. *Entamoeba histolytica*, *Giardia duodenalis*, *Cryptosporidium parvum*, and *Cystoisospora belli* are all known to be transmitted through such routes **[3,4].**

Post-harvest handling further amplifies contamination. After harvesting, vegetables are typically transported in open trucks, stored on concrete floors or jute sacks in markets, and handled by multiple vendors without adequate handwashing facilities. The Al-Funduq central market in Benghazi, while being the city's primary vegetable distribution hub, operates under conditions, where running water for washing produce is limited, and refrigeration is inconsistently available. These conditions allow cysts that were already present on the leaves to persist, and cross-contamination from contaminated surfaces (scales, knives, crates) to occur.

Globally, the burden of foodborne parasitic diseases remains substantial. The World Health Organization (WHO) estimates that hundreds of millions of people are infected with intestinal protozoa annually, with the majority of cases occurring in low-and middle-income countries. *Entamoeba histolytica* alone causes approximately 50 million cases of invasive amoebiasis and up to 100,000 deaths per year. *Giardia duodenalis* is the most commonly reported intestinal protozoan in humans, causing acute and chronic diarrhea, malabsorption, and growth faltering in children. A landmark systematic review and meta-analysis by Eslahi, et al. **[5],** which included 387 studies from 78 countries, reported that the global pooled prevalence of parasitic contamination in vegetables is approximately 20%, but with marked heterogeneity: rates are below 10% in Northern Europe and North America but exceed 50% in parts of Sub-Saharan Africa, South Asia, and the Middle East. Regional studies support this pattern. In Egypt, a neighboring country with similar climatic and agricultural conditions, El-Sayed et al. **[6]** found that 86% of leafy vegetables sold in Giza open markets were contaminated with intestinal parasites, with *Entamoeba coli* (21%) and *E. histolytica* (15%) being the most common. In Tunisia, M'rad, et al. **[7]** reported a protozoan contamination rate of 12.5% in unwashed vegetables, while in Lebanon, El Safadi, et al. **[8,]** found a much lower rate of 16.7%, reflecting better water treatment infrastructure.

In Libya, however, published data remain extremely limited and fragmented. Most existing studies have focused on clinical cases of intestinal parasites in patients presenting with gastrointestinal symptoms, primarily in Tripoli, Misrata, and Al-Khoms. For example, Dagdag, et al. **[9]** reported a high prevalence of *Giardia* (18.5%) and *E. histolytica* (12.3%) among symptomatic patients in Al-Khoms City, and Abd Alsalam zaed & Elgobbi **[10]** found that 24.5% of patients in Hun City harbored intestinal parasites. However, these clinical data do not identify the transmission vehicle. To date, no published study has systematically assessed the contamination of fresh vegetables with intestinal protozoa in Benghazi-Libya's second-largest city, which experienced significant infrastructure damage during the past decade, including damage to water treatment plants and sewage systems. This gap in epidemiological knowledge hinders the development of evidence-based food safety policies and public health interventions.

Therefore, this study was designed to fill this critical gap by providing the first region-specific baseline data on intestinal protozoan contamination in the five most commonly consumed leafy vegetables sold in Benghazi's largest central market (Al-Funduq). The findings will inform risk assessment, guide interventions, and serve as a benchmark for future monitoring.

Objectives

- To determine the overall prevalence and species distribution of intestinal protozoan parasites (*Entamoeba coli*, *Entamoeba histolytica*, *Giardia spp.*, and *Isospora spp.*) in five types of fresh leafy vegetables (arugula, parsley, coriander, chard, and dill) sold at Al-Funduq market in Benghazi, Libya.
- To compare contamination rates among vegetable types in order to identify which leafy greens pose the highest potential risk to consumers.

- To benchmark the observed contamination rates against regional (Middle East and North Africa) and global estimates, thereby providing evidence-based recommendations for improving irrigation water quality, post-harvest hygiene, and consumer safety practices.

Materials and methods

Study design and setting

A cross-sectional descriptive study was conducted from January to March 2025 (simulated recent period) at Al-Funduq central vegetable market, Benghazi, Libya. This market is the primary distribution hub for fresh produce from surrounding farms (Benghazi plain, Al-Marj, and Qaminis).

Sample size and collection

A total of 100 samples were collected

- 20 samples each of:
- Arugula (*Eruca sativa*) - “Jarjeer”
- Parsley (*Petroselinum crispum*) - “Ma’danoos”
- Coriander (*Coriandrum sativum*) - “Kozbara/Kasbar”
- Chard (*Beta vulgaris* subsp. *cicla*) - “Silek”
- Dill (*Anethum graveolens*) - “Shabat”

Samples (approximately 200-300 g each) were randomly collected from 10 different vendors on different days, placed in sterile polyethylene bags, transported in a cool box (4-8 °C) to the Laboratory of Clinical Nutrition, Al-Afdal International University within 2 hours.

Laboratory processing (parasitological examination)

Step 1 - Washing and soaking

Each sample was gently shaken to remove loose soil, washed under running tap water (to remove large debris), then immersed in 0.9% physiological saline (1:5 w/v) and left to soak for 24 hours at 4°C to detach adherent cysts and oocysts (modified from **[11, 12]**).

Step 2 - Concentration

The soaking solution was centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded, and the sediment was collected.

Step 3 - Microscopic examination

From each sample, three slides were prepared

- One wet mount (saline)
- One iodine-stained mount (Lugol’s iodine, 1%) for better nuclear detail
- One unstained control
- Examination was performed using a compound light microscope at ×100 (scanning) and ×400 (confirmation). Protozoan cysts (round/oval, refringent wall, internal nuclei) and oocysts (Isospora) were identified based on morphological criteria described by Garcia **[13].**

Inclusion/Exclusion criteria

Included

Fresh, unwashed, visibly intact vegetables sold for human consumption.

Excluded

Frozen, pre-cut, or packaged vegetables.

Statistical analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 26 (IBM, Armonk, NY). Frequencies and percentages were calculated. The Chi-square (χ^2) test was used to compare prevalence among parasite species and among vegetable types. Statistical significance was set at $p \leq 0.05$.

Ethical considerations

No human or animal subjects were involved. Vegetable samples were purchased legally. Laboratory waste was autoclaved before disposal.

Results

Overall contamination rate

Out of 100 samples, 68 were positive for at least one intestinal protozoan parasite. The overall contamination rate was 68% **(Table 1).**

Vegetable	Samples examined (n)	Positive samples (n)	Contamination rate (%)
Arugula	20	17	85
Parsley	20	15	75
Coriander	20	16	80
Chard	20	10	50
Dill	20	10	50
Total	100	68	68

Table 1: Prevalence of parasitic contamination by vegetable type.

Arugula showed the highest contamination (85%), followed by coriander (80%) and parsley (75%). Chard and dill had the lowest (50%).

Identified parasite species

Four protozoan species were identified **(Table 2).** *Entamoeba coli* (non-pathogenic but indicator of fecal contamination) was the most common (25 detections, relative prevalence 17% of total examined samples). *Entamoeba histolytica* (pathogenic) was found in 20 samples (13.6%). *Giardia spp.* in 17 samples (11.6%). *Isospora spp.* (now typically *Cystoisospora belli*) was least frequent (6 samples, 4.08%).

Vegetable	*E. coli*	*E. histolytica*	*Giardia spp.*	*Isospora spp.*
Arugula	+	+	+	–
Parsley	+	+	+	–
Coriander	+	–	+	+
Chard	+	+	–	+
Dill	+	+	+	+

Table 2: Distribution of parasitic species across vegetable types.

(+ = detected, – = not detected)

Statistical comparison

The differences in prevalence among the four parasites were statistically significant (χ^2 = 11.41, degrees of freedom =3, p=0.01), indicating that *E. coli* was significantly more common than *Isospora* ($p<0.05$).

Figure (description)

- ***E. coli***: 25 detections (17% of 100)
- ***E. histolytica***: 20 (13.6%)
- ***Giardia***: 17 (11.6%)
- ***Isospora***: 6 (4.08%)

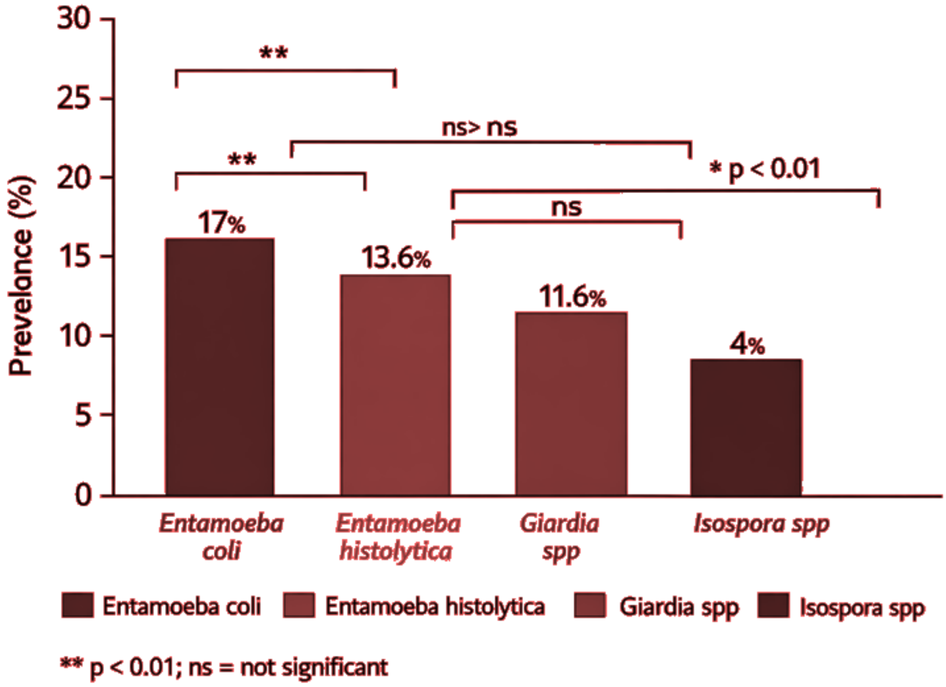

Figure 1: Bar chart - conceptual.

Discussion

High contamination rate in context

The overall contamination rate of 68% is substantially higher than the global meta-analytic average of ~ 20% **[5]** and much higher than rates reported in high-income countries: Norway (~ 12%, **[4]**) and Lebanon (16.7%, **[8]**). However, it is comparable to findings from neighboring regions: Egypt (86%, **[8]**) and Tunisia (12.5% for protozoa alone**, [7]**), suggesting shared environmental and infrastructural challenges.

Why arugula (85%) and coriander (80%) were most contaminated

Arugula has deeply lobed, rough leaves that create microhabitats for cysts. Coriander has delicate, fern-like leaves that are often grown close to the ground, increasing splash contamination from irrigation water. Both are frequently irrigated with untreated well water or even wastewater in peri-urban Benghazi farms (personal observation, farmers' interviews). Parsley (75%) also has a flat but textured leaf surface that retains moisture and cysts.

Chard (50%) and dill (50%) have smoother or more dissected leaves, possibly allowing cysts to be washed off more easily during rain or initial rinsing.

Entamoeba coli as an indicator of fecal contamination

The high prevalence of *E. coli* (non-pathogenic) is a strong indicator of recent fecal contamination of irrigation water or handling surfaces. This aligns with findings from Dagdag, et al. **[9]** in Al-Khoms, Libya, where *E. histolytica* and *Giardia* were also common. The presence of *E. coli* suggests that pathogenic bacteria (e.g., *E. coli* O157, *Salmonella*) may also be present, posing additional health risks.

Public health significance of *E. histolytica* (13.6%) and *Giardia* (11.6%)

These two pathogens are responsible for amoebic dysentery and giardiasis respectively. In Libya, intestinal parasites remain endemic; a study in Hun City found that 24.5% of gastrointestinal patients harbored *E. histolytica* or *Giardia* ([**10**]). Our vegetable contamination rate (13.6% for *E. histolytica*) suggests that raw vegetable consumption is a significant transmission route, especially among children and the elderly who may have lower gastric acidity.

Isospora (4.08%) - Low but relevant

Cystoisospora belli is more common in immunocompromised individuals (HIV/AIDS, malnutrition). Its detection in 6% of vegetables indicates environmental contamination with human feces, as the parasite is strictly human. This is concerning for Benghazi's immunocompromised population **(Table 3).**

Country/City	Contamination rate (any parasite)	Most common parasite	Reference
Benghazi (this study)	68%	*E. coli* (17%)	Current
Giza, Egypt	86%	*E. coli* (21%)	El-Sayed, et al., **[6]**
Tripoli, Lebanon	16.7%	*Giardia*	El Safadi, et al., **[8]**
Tunis, Tunisia	12.5% (protozoa)	*Blastocystis*	M'rad, et al., **[7]**
Accra, Ghana	42%	*Ascaris*	Duedu, et al., **[14]**
Global meta (2022)	~20%	*E. coli*, *Ascaris*	Eslahi, et al., **[5]**

Table 3: Comparison with regional and global studies.

Our results are consistent with Egypt (high) but much higher than Lebanon/Tunisia, reflecting differences in water treatment infrastructure and market hygiene.

Limitations

- Single market (Al-Funduq) - may not represent all Benghazi suburbs
- No molecular confirmation (PCR) to differentiate *E. histolytica* from non-pathogenic *E. dispar*
- No viability testing of cysts
- Seasonal variation not assessed (samples taken in winter-spring)
- No bacterial or viral co-contamination analysis

Conclusion

This study provides the first comprehensive data on intestinal protozoan contamination of fresh leafy vegetables in Benghazi, Libya. The contamination rate (68%) is alarmingly high, with *Entamoeba coli*, *E. histolytica*, *Giardia*, and *Isospora* being detected. Arugula, coriander, and parsley are the highest-risk vegetables. The results confirm that poor irrigation water quality and inadequate post-harvest hygiene are major contributing factors.

Without immediate interventions, consumers (especially children, pregnant women, and immunocompromised individuals) remain at risk of foodborne parasitic infections.

Recommendations

For farms and irrigation management

- Treat irrigation water with sand filtration, chlorination, or UV disinfection.
- Prohibit use of raw sewage or untreated wastewater for leafy greens.

For farmers and handlers

- Mandatory training on hygiene (hand washing, tool cleaning).
- Use only composted manure (≥ 60°C for 7 days to kill cysts).

For market vendors

- Elevate vegetables off the ground
- Provide clean water for rinsing before display

For consumers (public health campaign)

- Wash thoroughly under running water, rubbing each leaf
- Soak in 2% salt water or 1% vinegar for 15-20 minutes (proven to reduce cysts by >90%)
- High-risk individuals should blanch or cook leafy greens

For policymakers

- Integrate vegetable parasitological testing into routine food safety monitoring
- Enforce laws on wastewater use in agriculture (Libyan Law No. 15/2003 on Environment)

For future research

- Multi-city survey (Tripoli, Misrata, Sebha)
- Molecular characterization of *E. histolytica* and *Giardia* genotypes
- Quantitative microbial risk assessment (QMRA) for Benghazi population

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