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# Prevalence of intestinal parasites and microbial contamination in common edible vegetables used in Gaza Governorate, Palestine

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ABSTRACT

The present study was conducted to assess the extent of contamination with human intestinal parasites and to determine the bacterial quality in commonly vegetables in Gaza Governorate. A total of 320 vegetable samples which included dill, Jew's mallow, lettuce, parsley, peppermint, rocket, and spring onion were collected from the local markets in Gaza and examined for their contamination with human intestinal parasites and bacteria. These vegetables were processed by parasitological and microbial standard methods. The present study showed that 118 samples out of 320 were contaminated with human parasites with a prevalence rate of 36.9%. The lowest contaminated vegetable was spring onion at 3.43% and the highest contaminated one was Jew's mallow (6.87%). The most recovered parasite was the nematode *Strongyloides stercoralis*, which has a percent rate 17.2%. Coliform, fecal coliform, and *Escherichia coli* were detected in high percentage of the tested vegetables. Six vegetable samples were contaminated with *Cryptosporidium* sp. It is concluded that the fresh commonly used vegetables in Gaza governorate were contaminated with intestinal parasites and bacteria. Measures and inspection of the commonly used fresh vegetables by health authorities should be regular in Gaza community.

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## 1. Introduction

Worldwide, food borne diseases are major health burden leading to high morbidity and mortality. The global burden of infectious diarrhea caused by contaminated food and water involves 3-5 billion cases and nearly 1.8 million deaths annually, mainly in young children (1). According to the Control Disease Center an estimated 76 million cases of foodborne disease are reported annually in the United States with approximately 5000 deaths (2). The intestinal parasites are widely endemic in Gaza strip with different prevalence ranged from 25% to 53%, where many studies have been conducted in Gaza strip on school children, pre-school children, and in hospitals (3,4). The poor hygiene and deteriorated sanitation and environmental conditions that exist in Gaza strip may put at risk the contamination of vegetables by parasites due to the continuous flooding of sewers and using of suspected sludge as fertilizers in agriculture.

Studies have implicated how food can act as an important transmitter of enteropathogens. In Lebanon, raw ingredients for preparing meals and uncooked foods left at home have been reported to contain a range of bacteria, viruses and parasites, in 80% of vegetables and fruit specimens (5). Many vegetables are consumed fresh without cooking such as dill, lettuce, parsley, peppermint, rocket, and spring onion and these items are very important ingredients in the preparation of fresh vegetables salad in the local community in Gaza strip.

According to Alhindi and Mervat (6) vegetables and fruits from farms are transported usually to the internal community by donkeys especially in popular areas of Gaza strip, to be sold in local markets. Therefore, intestinal parasitic infection is

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easily transmitted from the contamination of these vegetables and fruits. The aim of this study was to determine the extent of contamination with intestinal parasites and to determine the microbial quality in the commonly used vegetables in Gaza governorate.

## 2. Materials and methods

320 vegetable samples were collected and the weight of each sample was 20 g. They were collected early morning from the three central local markets in Gaza Governorate (Feras, Al-Zawiya, and Al-Shagaaia markets). Seven vegetables were examined for the detection of human parasites and bacterial contamination. It included dill, Jew's mallow, lettuce, parsley, peppermint, rocket, and spring union, were collected between May, 2010 and December, 2011.

Each vegetable sample was washed with either water or Tris-buffered saline (TBS). This wash solution was centrifuged and the sediment was examined for parasites stages by direct smear microscopy under ×10, ×40. Each vegetable was divided into parts (leaves, middle parts, and roots). All parts were mixed and approximately 20-30 g was soaked in 50 ml of extraction solution in 150 ml beaker for 10 minutes. The washing solution (TBS, tap water) was poured through sterile gauze into cups and left to sediment for 15-20 minutes. After that, the supernatant was decanted and the sediment transferred into a 15 ml conical centrifuge to be centrifuged at 1500 rpm for 5 minutes.

The supernatant was decanted, and then a few drops of 10% formal saline were added to sediment. Few drops of sediment were placed on a microscopic slide and examined for parasites stages.

A smear for the sediment onto a slide was made, stained with Zeil–Neelsen staining and was examined under light microscope according to the procedure of the WHO (7).

One hundred and seventy five vegetable specimens were tested for their total coliform, fecal coliform, and *Escherichia coli* content using standard method Bacteriological Analytical Manual (8). In short, 25 g of each vegetable were aseptically added to 225 ml of phosphate buffered phosphate in a sterile stomacher bag. The mixture was homogenized for 5 minutes in a stomacher. Serial dilutions were made, and the total coliform count was performed by plating on violet red bile agar. Membrane fecal coliform agar was used to enumerate fecal coliforms and EC agar for *E. coli*. Confirmation was performed using API 20E (Biomerieux, France).

Each sample was examined for the presence of *Cryptosporidium* protozoan antigen using enzyme-linked immunosorbent assay (ELISA): Stool samples were processed according to manufacturer's recommendations (International Immuno-Diagnostics, Foster, USA, 2003). The required numbers of wells were prepared as needed (number of samples plus 2 for controls) and were placed in the holder. Two drops of negative control were added to well #1 and two drops of positive control to well #2. Two drops of stool supernatant were added to each test well. The plates were incubated for 30 minutes at room temperature and then washed. Two drops of reagent 1 were added to each well; then the plate was incubated for 5 minutes, then washed. Two drops of reagent 2 were added to each well, then the incubation step for 5 minutes, then washed. Two drops of chromogen were added to each well, then the incubation step for 5 minutes. Two drops of stop solution were added to each well. The wells were mixed by gently tapping the side of the strip holder with index finger. Absorbance was read at "wave length 450 nm" using Stat Fax ELISA reader. Data generated from the sample analysis were uploaded to Statistical Package for the Social Sciences (SPSS) (version 11, SPSS Inc., Chicago, IL, USA). Frequency and cross tabulation were performed. The two variables were determined; vegetables as independent variable and the contamination with parasites or microbes as dependent variables.

## 3. Results

In this study, seven types of vegetables were collected (n = 320) and examined for the presence and contamination with human intestinal parasites, and 175 of those samples were further examined for microbial contamination. The microscopic examination using TBS and sedimentation techniques revealed that 118 out of 320 were contaminated with various human intestinal parasites with a prevalence rate of 36.9%.

As shown in table 1, all seven types of vegetables are contaminated with intestinal parasites; the lowest contaminated vegetable was spring onion (9.3%). High prevalence for intestinal parasites contamination was noticed for Jew's mallow (18.6%). There was no statistically significant difference between the contamination percentage rates of different vegetable types ($\chi^2 = 5.680$, $P = 0.460$). When using ELISA for antigen detection, *Cryptosporidium* sp. was found contaminating two vegetable types; *Anethum graveolens* and *Erica sativa* with a prevalence of 9.3% and 5.0%, respectively. All other types of vegetables were found negative for *Cryptosporidium* antigen as shown in table 1.

Three protozoa, *Entamoeba histolytica/dispar* (3.0%), *Giardia lamblia* (1.5%), and *Cryptosporidium* species (1.5%) were detected.

**Table 1.** Prevalence rate of human intestinal parasites stages contaminating the seven types of vegetables in Gaza Governorate markets (n = 320)

| Vegetable | Common name | Methods of diagnosis | Number of samples examined | Number contaminated | % |
|---|---|---|---|---|---|
| *A. graveolens* | Dill | Wet mount using saline | 43 | 13 | 11.0 |
| *C. olitorius* | Jew's mallow | | 49 | 22 | 18.6 |
| *L. sativa* | Lettuce | | 50 | 20 | 16.9 |
| *P. crispum* | Parsley | | 48 | 21 | 17.8 |
| *M. piperita* | Peppermint | | 48 | 16 | 13.6 |
| *E. sativa* | Rocket | | 40 | 15 | 12.8 |
| *A. fistulosum* | Spring onion | | 42 | 11 | 9.3 |
| Total | | | 320 | 118 | 100 |
| *A. graveolens* | | Antigen detection | 43 | 4 | 9.3 |
| *E. sativa* | | | 40 | 2 | 5.0 |
| Total | | | 83 | 6 | 14.3 |

*C. olitorius*: *Corchorus olitorius, L. sativa*: *Lactuca sativa, P. crispum*: *Petroselinum crispum, M. piperita*: *Mentha piperita, E. sativa*: *Erica sativa, A. fistulosum*: *Allium fistulosum, A. graveolens*: *Anethum graveolens*

In addition to three helminthes, *Ascaris lumbricoides* (1.5%), *Enterobius vermicularis* (1.5%), and *Strongyloides stercoralis* (83.3%) were detected. The most common nematodes revealed was *S. stercoralis* larvae where 55 vegetable samples were positive to this parasite as shown in table 2.

**Table 2.** Parasite species and stages detected in vegetables samples collected from three common markets in Gaza Governorate (number of samples examined = 320)

| Parasites | Stage/s detected | Number of positive samples | % |
|---|---|---|---|
| *E. histolytica/dispar* | Cyst | 2 | 3.0 |
| *G. lamblia* | Cyst/trophozoite | 1 | 1.5 |
| *Cryptosporidium* sp. | Oocyst | 6 | 9.2 |
| *A. lumbricoides* | Egg | 1 | 1.5 |
| *E. vermicularis* | Egg | 1 | 1.5 |
| *S. stercoralis* | Larva | 55 | 83.3 |
| Total | | 66 | 100 |

*E. histolytica/dispar*: *Entamoeba histolytica/dispar, G. lamblia*: *Giardia lamblia, A. lumbricoides*: *Ascaris lumbricoides, E. vermicularis*: *Enterobius vermicularis, S. stercoralis*: *Strongyloides stercoralis*

Table 3 summarizes the level of contamination with *S. stercoralis* among the studied vegetables. Parsley was the highest contaminated one with a prevalence of 23.6% followed by Jew's mallow 21.8%, while Dill, rocket, and spring onion have similar prevalence's 12.7%.

**Table 3.** Percentage contamination rates with *S. stercoralis* among various types of vegetables collected from local markets in Gaza Governorate

| Vegetable | Number of samples examined | Number contaminated | % |
|---|---|---|---|
| *A. graveolens* | 43 | 7 | 12.7 |
| *C. olitorius* | 49 | 12 | 21.8 |
| *L. sativa* | 50 | 6 | 11.0 |
| *P. crispum* | 48 | 13 | 23.6 |
| *M. piperita* | 48 | 3 | 5.5 |
| *E. sativa* | 40 | 7 | 12.7 |
| *A. fistulosum* | 42 | 7 | 12.7 |
| Total | 320 | 55 | 100 |

$\chi^2$ = 10.153, P = 0.118. *A. graveolens*: *Anethum graveolens, P. crispum*: *Petroselinum crispum, M. piperita*: *Mentha piperita, A. fistulosum*: *Allium fistulosum, C. olitorius*: *Corchorus olitorius, L. sativa*: *Lactuca sativa, E. sativa*: *Erica sativa*

Coliform, fecal coliform and *E. coli* were the bacterial indicators tested for the vegetables as shown in table 4. *E. coli* and fecal coliform were highest in parsley. Mostly, all seven vegetables were positive and contaminated with coliform. The present study revealed that coliform was found in 174 out of 175 of vegetables (99.44%) followed by fecal coliform 64 (36.5%) and *E. coli* 54 (30.8%).

**Table 4.** Microbial contamination of seven vegetables from Gaza Strip markets (n = 175)

| Vegetable | n | *E. coli* positive n (%) | Fecal coliform positive n (%) |
|---|---|---|---|
| *A. graveolens* | 25 | 12 (48) | 13 (52) |
| *C. olitorius* | 25 | 3 (12) | 14 (56) |
| *L. sativa* | 25 | 2 (8) | 2 (8) |
| *P. crispum* | 25 | 16 (64) | 15 (60) |
| *M. piperita* | 25 | 6 (24) | 6 (24) |
| *E. sativa* | 25 | 9 (36) | 8 (32) |
| *A. fistulosum* | 25 | 6 (24) | 6 (24) |
| Total | 175 | 54 (30.8) | 64 (36.5) |

*A. graveolens*: *Anethum graveolens, P. crispum*: *Petroselinum crispum, M. piperita*: *Mentha piperita, A. fistulosum*: *Allium fistulosum, C. olitorius*: *Corchorus olitorius, L. sativa*: *Lactuca sativa, E. sativa*: *Erica sativa, E. coli: Escherichia coli*

## 4. Discussion

The majority of the epidemiological information on fruits and vegetables as vehicles of foodborne disease comes from very few countries. In this study, vegetables collected from Gaza markets were found to be contaminated with one or more intestinal parasites, with 36.9% overall prevalence. These findings could be an indicator for the severity of the problem of intestinal parasitic diseases in Gaza strip. In the Palestinian community, these vegetables are used in preparing salad and/or eaten fresh (except Jew's mallow) as in other places. The prevalence was low compared to the results reported in Tehran (65%) (9). In another study in Nigeria, vegetables were more contaminated than fruits (71.2% vs. 28.8%). From 118 helminths ova isolated, 64 (54.2%) were *Ascaris lumbricoides*, 11 (9.3%) *Trichuris trichiura,* and 43 (36.4%) hookworm (10).

The findings obtained in the present study indicated that all seven vegetables were contaminated with similar intestinal parasites; this may be explained by the possible exposure of vegetables to similar contaminating sources like sewage flooding near farms or using fresh animal manure as fertilizers. Alhindi and Mervat (6) reported that the continuous flooding of sewers in Gaza is a problematic issue due to over pressure on the sewers system in the area. This puts people at risk of Ascariasis infection and other parasites.

In this study, two protozoan parasites; *E. histolytica/dispar* cyst and *G. lamblia* cyst; and three types of nematodes; *A. lumbricoides, E. vermicularis,* and *S. stercoralis* were reported. Most literature in Gaza strip reported the same types of intestinal parasites which are considered endemic in the community (3,11). Hence, these two protozoans seem to be the most common ones in the Palestinian community of Gaza strip. *E. histolytica/dispar*, *G. lamblia*, *A. lumbricoides*, *T. trichiura*, *S. stercoralis*, *E. vermicularis*, *H. nana,* and *T. saginata* were reported in the health records of the Palestinian Ministry of Health (12). In Tripoli-Libya, of the 36 tomato, 36 cucumber, 27 lettuce, and 27 cress samples examined eggs of *Ascaris* spp. were detected in 19%, 75%, 96%, and 96%, respectively (13).

The present findings showed that *S. stercoralis* was the highest prevalence (17.2%) among all other detected parasites. The life cycle of this parasite and the presence of the larval stages in the soil facilitate the contamination of vegetables. In Saudi Arabia, the prevalence of the parasites was 28% in green onion, 25% in radish, 17% in watercress, 17% in lettuce, and 13% in leek. The most parasites detected were *Ancylostoma duodenale, Entamoeba coli, A. lumbricoides,* and *Blastocystis hominis* (14). The highest contaminated vegetables with *S. stercoralis* were Jew's mallow (24.5%) and Parsley (27.1%), respectively. Peppermint had the lowest contamination percentage (6.3%). This may be due to its contents of menthol. People usually eat fresh vegetables in local markets, unaware of the dangers of being contaminated with parasites. In addition, the extent of awareness of the local community regarding foodborne diseases. A study on vegetables from kitchen gardens in Campinas, Brazil was conducted. The bacterial analysis condemned 22.3% of the vegetable samples, and the parasitological examination condemned 14.5% (15). Helminths egg contamination of vegetables purchased at suburban market in Hanoi, Vietnam was examined where (n = 317) and 82 (26%) were shown to be positive for parasite eggs. Contamination was highest in leafy vegetables (31%), followed by root vegetables (17%) and fruits (3%). Throughout the survey, five species of parasite eggs were found: *Ascaris* sp., *Trichuris* sp., *Toxocara* sp., *Taenia* sp., and *Ascaridia galli* (16).

In this study, *Cryptosporidium* sp. was found in two vegetables; Dill and Rocket with a total prevalence of 3.4%. In 2005, an outbreak of diarrhea affected company employees near Copenhagen. In all 99 employees were reported ill; 13 were positive for *Cryptosporidium hominis* infection. Disease was associated with eating from the canteen salad bar on one, possibly two, specific weekdays (17).

Most *E. coli* are not pathogenic and are part of the normal human and animal gut flora. This supports the hypothesis that vegetable positive for these organisms are fecally contaminated. The enterovirulent *E. coli* include seven groups believed to be associated with diarrhea (18).

The possible contamination risk factors of vegetables in our community are as follows: flooding of sewage, using fresh animal manure, small-scale reuse of waste water in farms, the contamination by vendors on caros (horses or donkeys drawn wagon), zoonotic transmission, and cross-contamination between transported vegetables.

## 5. Conclusion

The commonly used vegetables in Gaza governorate were found contaminated with intestinal parasites and bacteria. The contamination found will constitute a high risk for human. *S. stercoralis* should be considered and urgent actions should be taken to ensure the quality vegetables in markets. Establishments of regulations to improve avoiding risk factors for vegetables contamination. Inspection and examination of the used vegetables by health authorities should be regular. Education of people regarding foodborne diseases and methods of transmission and contamination by pathogens is required.

## Conflict of Interests

Authors have no conflict of interest.

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## References

1. World Health Organization (WHO). 2007. Food safety and foodborne illness. Fact Sheet N°237. WHO.
2. Centers for Disease Control (CDC). Food-borne diseases. CD Alert, Monthly Newsletter of National Centre for Disease Control, Directorate General of Health Services, Government of India. 2009; 13(4): 1-12.
3. AL-Zain B, Al-Hindi A. Distribution of Strongyloides stercoralis and other intestinal parasites in household in

Beit-lahia city, Gaza Strip, Palestine. Annals of Alquds Medicine 2005; 1: 48-52.
4. Al-Hindi A. Diagnosis of gastrointestinal parasites among hospitalized patients attending Al-Nasser Paediatric Hospital, Gaza, Palestine. J Public Health 2009; 17: 49-53.
5. Abdelnoor AM, Batshoun R, Roumani BM. The bacterial flora of fruits and vegetables in Lebanon and the effect of washing on the bacterial content. Zentralbl Bakteriol Mikrobiol Hyg B 1983; 177: 342-349.
6. Alhindi AI, Mervat AL. Trends of intestinal parasites prevalence in the Gaza Strip, 1998-2007: The use of government health records. Turk J Med Sci 2013; 43: 652-659.
7. World Health Organization (WHO). 1994. Bench aids for the diagnosis of intestinal. WHO.
8. United State Food and Drug Administration (FDA). 2015. Bacteriological Analytical Manual (BAM). FDA. 946p.
9. Gharavi M, Jahani MR, Rokni MB. Parasitic contamination of vegetables from farms and markets in Tehran. Iran J Public Health 2002; 31: 83-86.
10. Uneke CJ. Potential for geohelminth parasite transmission by raw fruits and vegetables in Nigeria: Implication for a risk profile. J Nutr Environ Med 2007; 16: 59-68.
11. Kanoa B, George E, Abed Y, et al. Evaluation of the relationship between intestinal parasitic infection and health education among school children in Gaza city, Beit-lahia village and Jabalia refugee camp, Gaza strip, Palestine. Islamic Univ J 2006; 14: 39-49.
12. Ministry of Health. Health status in Palestine-The Annual Report 2005. Gaza, Palestine: Health Information Centre, Ministry of Health. 2006.
13. Abougrain AK, Nahaisi MH, Madi NS, et al. Parasitological contamination in salad vegetables in Tripoli-Libya. Food Control 2010; 21: 760-762.
14. Al-Binali AM, Bello CS, El-Shewy K, et al. The prevalence of parasites in commonly used leafy vegetables in South Western, Saudi Arabia. Saudi Med J 2006; 27: 613-616.
15. Simoes M, Pisani B, Marques EGL, et al. Hygienic-sanitary conditions of vegetables and irrigation water from kitchen gardens in the municipality of Campinas, SP. Braz J Microbiol 2001; 32: 331-333.
16. Uga S, Hoa NT, Noda S, et al. Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam. Nepal Med Coll J 2009; 11: 75-78.
17. Ethelberg S, Lisby M, Vestergaard LS, et al. A foodborne outbreak of Cryptosporidium hominis infection. Epidemiol Infect 2009; 137: 348-356.
18. European Commission. Risk profile on the microbiological contamination of fruits and vegetables eaten raw. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out125_en.pdf. Cited: Apr 29, 2002.