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# Parasitic stages contaminants of some vegetables selected from super and local markets of Kirkuk city, Iraq

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## ABSTRACT
Vegetables are essential components of a healthy human diet but their raw administration is an important means of transmitting many infectious diseases. From October 2018 to May 2019, this study was intended to detect the parasitic pollutants in some common green vegetables used for fresh intake in Kirkuk city's super and local markets. On the basis of Kirkuk city's geographical division, four areas were selected: Rahim-awa, Huzairan-one, Wassiti and Ras Al-JisrJisr. Eight types of vegetables from each region were collected including tomato, lettuce, cucumber, radish, celery, leek, cabbage and basil. Parasitic stages were microscopically detected by direct smear or by Iodine staining or modified Zeihl-Neelsen stain. Many parasitic stages (eggs, oocysts, and cysts) have been found contaminating the vegetables. In general vegetables were more contaminated with parasitic stages in local markets than in supermarkets. *Entamoeba histolytica* was the most common parasite detected at 21.1 % frequency. Followed by *Giardia lamblia, Cryptosporidium parvum, Hyminolepis nana, Echinococcus granulosus and Microsporidia sp.*, with 12.8 %, 5.4%, 4.2%, 3.5 % and 2.3 % for all parasites respectively. Radish, celery and leek were more commonly contaminated than cucumber, tomato, lettuce, basil and cabbage. In Ras Al-Jisr, the highest rate of contamination was detected, followed by Huzairan- one and Wassiti, the lowest rate was detected in Rahim-awa. The conclusion is that, vegetables are more contaminated with parasitic stages in Kirkuk's local markets than in supermarkets. Parasites were found contaminating radish, celery, and leek more than cucumber, tomato, lettuce, basil and cabbage. The greatest isolated pathogenic parasites were the *Entamoeba histolytica* and the *Giardia lamblia* cysts. Multiple contaminated parasites have been observed in almost all vegetables.

**Key words:** Vegetables, Parasitic contaminants, Kirkuk city, Iraq.

## Introduction
One public human disease is parasitic infection. Around two billion persons have been found to be infected with parasites worldwide [1]. Oral ingestion is the primary means of transmitting a high number of parasite pathogens, particularly intestinal parasites [2]. Fresh vegetables can work as vehicles for the transmission of many types of parasites to humans [3]. Vegetables are part of the daily human diet because they contain a high amount of minerals, fibers, vitamins and other nutrients [4]. Despite vegetables that are important to the human body, they are part of the route to transmit various human pathogens, particularly if they are consumed fresh, uncooked or unwashed properly [5, 6, 7]. Recently, cases of foodborne infection have increased, primarily correlated with consumption of fresh vegetables[5, 6, 7, 8]. Millions of cases of severe infections and deaths in developing countries were linked to parasites [1]. Poor hygiene, inadequate health system and contamination are responsible for high parasites among people in some countries [6, 9, 10,11]. Several surveys from diverse parts of the world showed that, polluted vegetables are transmitters of parasitic protozoan cyst and/or oocysts of parasites such as *Entamoeba*, *Giardia*, *Cyclospora*, *Cryptosporidium*, *Isospora* and *Toxoplasma*. They also may transmit ova and/or larvae of parasitic helminthes such as *Teania*, *Hymenlepis*, *Toxocara*, *Fasciola*, *Trichostrongylus*, *Strongyloide*s, *Ascaris* and Hookworm [5, 6, 11]. This study intended to detect the parasitic pollution in some public vegetables used for fresh intake from Kirkuk city super and local markets.

## Materials and methods
**Sample collection**: 256 vegetable samples were collected from various sites in Kirkuk city by simple random selection during the period October 2018 to May 2019. Four neighborhoods were selected based on Kirkuk city's geographical division: Rahim-awa, Huzairan-one, Wassiti and Ras Al-Jisr regions. Eight fresh vegetable types were selected at each super and local market in each location. The vegetables

included: tomato *Solanum lycopersicum*, lettuce *Lactuca sativa*, cucumber *Cucumis sativus*, radish *Raphanus sativus*, celery *Apium graveolens*, leek *Allium ampeloprasum*, cabbage *Brassica oleracea* and basil *Ocimum basilicum*. About 250-500 gm. of vegetables were carried in separate nylon bags to the laboratory for examination.

**Vegetable processing**: About 200 g of edible parts of the vegetables were prepared after carefully examining the vegetables with a naked eye and cleaning them from the mud by immersing them in distilled water for 6-7 min. By shaking vigorously in clean glass bottles containing 200 ml of normal saline (NaCl 0.85% ), the cleaned weighted parts were washed to detach the parasite stages (protozoan cysts and oocysts, helminthic ova and larvae). Washing saline was sieved through eight layers of gauze to eliminate unwanted large particles, and then centrifuged at 5000 rpm for 5 minutes. The supernatant was drained off, and two methods were used to examine the residue. This procedure was repeated until all of the washing water had been completed [6].

**Parasite examination:**

**Wet mount direct smear**: A clean slide was applied with two small drops of the sediment. The droplets were mixed with a small drop of normal saline (NaCl 0.85% ) or with Iodine solution. The Iodine solution was prepared by mixing 2gm. of Potassium iodide with 1gm. of Iodine and dissolved in1000 ml of distilled water. The preparations were examined under a compounded light microscope, using 40x objective [10].

**Modified Ziehl-Neelsen stain**: Smears from the sediment were prepared, air dried and fastened with absolute methanol (96-99%). The smears were stained with the Kinyoun Carbol Fuchsin, then decolorized in acid alcohol, and stained with Malachite green, washed thoroughly, air-dried and finally examined for oocyst detection of *Cryptosporidium*, *Cyclospora* and *Microsporidia* using x40 and 100 x objectives. Egg albumin was used as an adhesive [12].

## Results

The examination of vegetables collected from super and local markets showed that, these vegetables were contaminated with many parasitic stages (eggs, oocysts and cysts) and non-parasitic organisms (whole insects, insects larva, flagellated and ciliated protozoa), as indicated in Table 1.

**Table 1: Detected parasitic and non-parasitic organisms in vegetables selected from Kirkuk super and local markets**

| Total examined vegetable samples | Local markets | | | Super markets | | |
|---|---|---|---|---|---|---|
| | Total No. examined | No. +ve parasites | No. +ve non-parasites | Total No. examined | No. +ve parasites | No. +ve non-parasites |
| 256 | 128 | 48 | 85 | 128 | 25 | 67 |
| Percentages | | 37.5 | 66.4 | | 19.5 | 52.3 |

In general, compared to the supermarkets, vegetables collected from local markets carried more parasitic and non-parasitic pollutants. As shown in table 2, the radish, celery and leek were more contaminated compared to the other screened vegetables.

**Table 2: Detected parasitic and non-parasitic stages in local and super markets according to vegetables type**

| Vegetable types (examined No.) | Local markets | | Super markets | |
|---|---|---|---|---|
| | No. +ve Parasites (%) | No. +ve non-parasites (%) | No. +ve parasites (%) | No. +ve non-parasites (%) |
| Lettuce (32) | 7 (14.5) | 16 (18.8) | 3 (12) | 11 (16) |
| Tomates (32) | 0 (0) | 3 (3.5) | 0 (0) | 0 (0) |
| Cucumber (32) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Radish (32) | 10 (20.8) | 16 (18.8) | 5 (20) | 15 (22.3) |
| Celery (32) | 10 (20.8) | 16 (18.8) | 8 (32) | 12 (17.9) |
| Leek (32) | 12 (25) | 16 (18.8) | 5 (20) | 15 (22.3) |
| Cabbage (32) | 0 (0) | 2 (2.3) | 0 (0) | 0 (0) |
| Basil (32) | 9 (18.7) | 16 (18.8) | 4 (16) | 14 (20.8) |
| Total No. (256) | 48 | 85 | 25 | 67 |

As presented in Table 3, *Entamoeba histolytica* was the most common parasite detected at 21.1 percent frequency. Followed by *Giardia lamblia*, *Cryptosporidium parvum*, *Hyminolepis nana*, *Echinococcus granulosus* and *Microsporidia sp*., with 12.8, 5.4, 4.2, 3.5 and 2.3 percent for all parasites respectively.

**Table 3: Frequency of detected parasites in examined vegetables**

| No. examined vegetables | Type of parasite | | | | | |
|---|---|---|---|---|---|---|
| | *E. histolytica* | *C. Parvum* | *Microsporidia sp.* | *G. Lamblia* | *E. granulosus* | *H. nana* |
| 256 | 54 | 5 | 2 | 33 | 9 | 11 |
| Percentage | 21.1 | 5.4 | 2.3 | 12.8 | 3.5 | 4.2 |

The highest rate of contamination was detected in Ras Al-Jisr markets. High rates were also recognized in Huzairan-one and Wassiti, while the lowest rate of contamination was in Rahim-awa markets. In addition, several contaminant species have been observed in all types of investigated vegetables, as shown in Table 4 and Figure 1.

**Table 4: Detected parasites type in studied regions**

| Examined regions | Type of detected parasite | | | | | |
|---|---|---|---|---|---|---|
| | *E. histolytica* | *C. Parvum* | *Microsporidia sp.* | *G. Lamblia* | *E. granulosus* | *H. nana* |
| Huzairan-one | 12(18.8%) | 2(3.1%) | 0 | 5(7.81%) | 2(3.1%) | 3(4.7%) |
| Rahim-awa | 10 (15.6%) | 0 | 0 | 6 (9.4%) | 1 (1.6%) | 2(3.1%) |
| Wassiti | 14 (21.9%) | 0 | 0 | 9(14.1%) | 4(6.3%) | 3(4.7%) |
| Ras Al-Jisr | 18 (28.1%) | 2 (3.2%) | 2 (3.2%) | 13(20.3%) | 2 (3.2%) | 3(4.7%) |

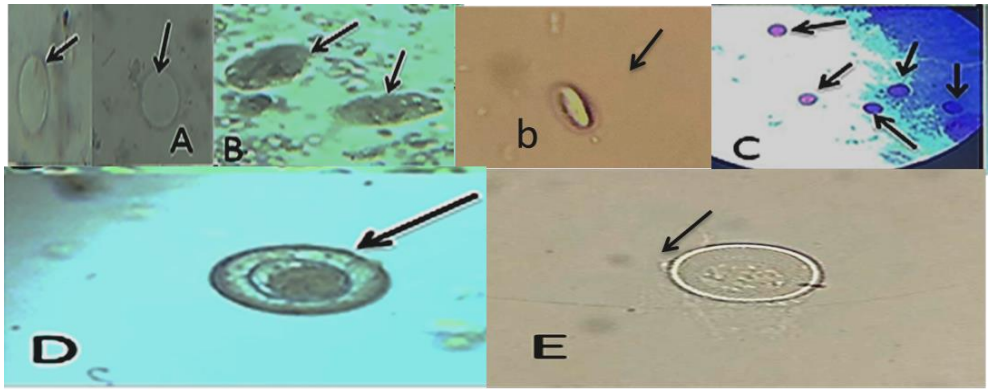


**Figure 1:A (cysts of *E. histolytica*), B, b (cysts of *G. lamblia*), C (oocysts of *C. parvum*), D (egg of *H. nana*), E (egg of *E. granulosus*).**

Results in table 5 showed that the most frequently detected parasite in Huzairan-one vegetables were *E. histolytica*, *G. lamblia*, *C. parvum*, while the rest were either low in prevalence or absent. For example, most of the contamination was from radish, celery and leek.

**Table 5: Detected parasites according to vegetables type in Huzairan-one markets**

| No. examined vegetables | Type of parasite | | | | | |
|---|---|---|---|---|---|---|
| | *E. histolytica* | *C. Parvum* | *Microsporidia sp.* | *G. Lamblia* | *E. granulosus* | *H. nana* |
| Lettuce (8) | 3 | - | - | 1 | - | - |
| Tomates (8) | - | - | - | - | - | - |
| Cucumber (8) | - | - | - | - | - | - |
| Radish(8) | 3 | 1 | - | 2 | 1 | 1 |
| Celery(8) | 2 | 1 | - | 1 | 1 | 1 |
| Leek (8) | 3 | - | - | 1 | - | 1 |
| Cabbage(8) | - | - | - | - | - | - |
| Basil(8) | 1 | - | - | - | - | - |
| Total samples (64) | 12(18.8%) | 2(3.1%) | 0 | 5(7.81%) | 2(3.1%) | 3(4.7%) |

Consequences in the Rahim-awa markets were not fluctuated from those recorded in the Huzairan-one region, as revealed in Table 6. Wherein *E. histolytica* showed highest frequency, with a rate of 15.6 % followed by *G. lamblia* and *H. nana*.

**Table 6: Detected parasites according to vegetables type in Rahim-awa markets**

| No. examined vegetables | Type of parasite | | | | | |
|---|---|---|---|---|---|---|
| | *E. histolytica* | *C. Parvum* | *Microsporidia sp.* | *G. Lamblia* | *E. granulosus* | *H. nana* |
| Lettuce (8) | 1 | - | - | 2 | - | - |
| Tomates (8) | - | - | - | - | - | - |
| Cucumber (8) | - | - | - | - | - | - |
| Radish(8) | 3 | - | - | 2 | - | - |
| Celery(8) | 2 | - | - | 1 | 1 | 1 |
| Leek (8) | 3 | - | - | 1 | - | 1 |
| Cabbage(8) | - | - | - | - | - | - |
| Basil(8) | 1 | - | - | - | - | - |
| Total samples (64) | 10 (15.6%) | 0 | 0 | 6 (9.4%) | 1 (1.6%) | 2(3.1%) |

The same occurrence was also noted in Wassiti region, where *C. parvm*, *G. lamblia*, *E. histolytica* were predominated, in radish, celery and leek, as illustrated in table 7.

**Table 7: Detected parasites according to vegetables type in Wassiti markets**

| No. examined vegetables | Type of parasite | | | | | |
|---|---|---|---|---|---|---|
| | *E. histolytica* | *C. Parvum* | *Microsporidia sp.* | *G. Lamblia* | *E. granulosus* | *H. nana* |
| Lettuce (8) | 2 | - | - | 1 | - | - |
| Tomates (8) | - | - | - | - | - | - |
| Cucumber (8) | - | - | - | - | - | - |
| Radish(8) | 2 | - | - | 3 | 2 | 1 |
| Celery(8) | 4 | - | - | 2 | - | - |
| Leek (8) | 5 | - | - | 3 | 2 | 2 |
| Cabbage(8) | - | - | - | - | - | - |
| Basil(8) | 1 | - | - | - | - | - |
| Total samples (64) | 14 (21.9%) | 0 | 0 | 9(14.1%) | 4(6.3%) | 3(4.7%) |

In Ras Al-Jisr markets, as displayed in Table 8, *E. histolytica* had the highest incidence compared to the other studied regions, with a percentage rate of 28.1%. Accordingly to *G. lamblia*, *H. nana*, each with 20.3 and 4.7 percent, respectively.

**Table 8: Detected parasites according to vegetables type in Ras Al-Jisr markets**

| No. examined vegetables | Type of parasite | | | | | |
|---|---|---|---|---|---|---|
| | *E. histolytica* | *C. Parvum* | *Microsporidia sp.* | *G. Lamblia* | *E. granulosus* | *H. nana* |
| lettuce (8) | 5 | - | - | 2 | - | - |
| Tomates (8) | - | - | - | - | - | - |
| Cucumber (8) | - | - | - | - | - | - |
| Radish(8) | 4 | 1 | 1 | 4 | 1 | 1 |
| Celery(8) | 3 | 1 | - | 2 | 1 | 1 |
| Leek (8) | 4 | - | 1 | 3 | - | 1 |
| Cabbage(8) | - | - | - | 1 | - | - |
| Basil(8) | 2 | - | - | 1 | - | - |
| Total samples (64) | 18 (28.1%) | 2 (3.2%) | 2 (3.2%) | 13 (20.3%) | 2 (3.2%) | 3 (4.7%) |

## Discussion

Vegetables can carry or facilitate pathogen transmission. Parasitic stage of protozoa and hemlines is primarily transmitted by fecal-oral means, especially of the intestinal type [2, 6].Vegetables are instrumental in shaping the epidemiology and transmission of parasites [3]. Food-borne illnesses and the involvement of fresh vegetables in the transmission of various types of parasites have been observed in recent years [13, 14, 15, 16, 17]. The current study isolated many types of parasitic stages from vegetables collected from super and lacal markets. This has agreed with a number of studies around the world and in Iraq [18, 19, 20, 21, 22]. Our results showed that the most common parasite found in the examined vegetables was *E. histolytica* followed by *G. lamblia*, *C. parvum* and *H. nana* whereas *Ascaris lumbricoides* was not detected. This result was agreed with studies in Iraq which recorded a high prevalence of *Entamoeba* and *Giardia* [16, 23] and was similar to those found by Al-Shawa [18], in which several intestinal parasites including *E. histolytica* and *G. lamblia* were isolated from vegetable samples. Also agreed with a similar study in Riyadh, Saudi Arabia, where parasite stages recovered from various green plants were *E. histolytica* was 35.5%, *G. lamblia* was 31.6% and *Hymenolepis sp*. was 14.5% [19]. But this result was in contrast to a study in South West Saudi Arabia which verified that *A. lumbricoides* eggs and *E. histolytica* cysts to be the most frequent parasites, in the five leafy investigated vegetables [15], and with Ismail's study whom recorded *Toxocara spp*, as the most helminthic isolates in Jordanian vegetables [17]. This variances in the detected parasitic types may be due to, the difference in parasites endemicity, climate and environmental factors present in each region that determines the existence and distribution of these species in each region. On the other hand, contamination of vegetables with parasitic stages may be originated from the use of wastewater for vegetables irrigation [24]. In contrast to untreated water, where they detected many types of parasites, no helminthes or growing parasite stages were found in the treated water [25]. Similarly, studies have shown that the main reason for the elevation of intestinal parasite frequency is the waste-farming and sewage water [19, 21]. In the present study, we noticed that the parasites in radish, celery and leek were more frequent than lettuce, basil, cabbage, cucumber and tomatoes, which was granted with a similar study [24]. The morphology and arrangement of the leaves, direct soil contact may contribute to differences in the rate of contamination seen in various vegetables. Studies also revealed that the existence of trichomes on the surface of the leaves can facilitate the adhesion of eggs, cysts and larvae [23 ]. And that overlapping mint leaves can protect helminthic eggs against drying and adverse conditions [20]. However, the smooth surface of some vegetables such as tomatoes and cucumber may decrease the degree of parasite attachment later on [12]. The parasites laden with leafy vegetable contamination were studied in Saudi Arabia, which revealed that the prevalence of parasites was 25% in radish, 17% in lettuce and 13% in leek [19]. While in

Gaza the prevalence of parasites was 17.5 percent in parsley, 13.7 percent in red cabbage and 12.5 percent in cucumber [8]. There was also a high incidence of parasites in the edible vegetables in the Southern of Saudi Arabia [15]. The present study revealed that the highest rate of contamination was in Ras Al-Jisr region, a higher rate was also detected in Huzairan-one and Wassiti regions, while Rahim-awa had the lowest rate of contamination. Factors such as: type of used irrigation water and vegetables after harvesting and handling methods, residents associated with sanitary behaviors [26], could be attributed to the wide prevalence range in many regions. The results of the current study also revealed a higher rate of contamination in local markets than in supermarkets, which could be due to the process of washing and cleaning these vegetables before they were brought to the supermarkets. In this study, several types of pollutants were seen in totally examined vegetables. This could specify the option of high-level of vegetable pollution, which could potentially mark many human parasite diseases.

## Conclusion

The conclusion is that the vegetables in Kirkuk local and super markets are contaminated with many types of parasitic stages, the contamination rate in local markets is greater than in super markets therefore, the vegetables are better to be washed properly before consumption and if vegetables have to be consumed, it is better to buy them from super markets.

**Data availability**

The datasets generated during the current study are available directly in this manuscript. The pre-collected data analyzed during this study are available from corresponding author and no restrictions apply to the availability of these data.

## Conflicts of Interest

The authors declare no conflict of interest.

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## الملخص

تعتبر الخضراوات جزء أساس من حمية الانسان الصحية بسبب قيمتها الغذائية ومع ذلك تناولها نيئة يمثل وسيلة مهمه لانتقال العديد الامراض المعدية. أجريت هذه الدراسة من تشرين الأول 2018 الى أيار 2019 للكشف عن التلوث الطفيلي في بعض الخضراوات الخضرية الشائعة والتي اعتيد على تناولها بشكل نيء في أسواق ومحال مدينة كركوك. اختيرت أربع احياء حسب التقسيم الجغرافي لمدينة كركوك والتي تشمل الاحياء رحيم اوة وواسطي وواحد حزيران وراس الجسر. ثمان عينات جمعت بشكل عشوائي من كل منطقة وشملت الطماطم والخس والخيار والفجل والكرفس والملفوف والريحان. استندت هذه الطريقة على كشف اطوار الطفيلي من الخضراوات عن طريق المسح المباشر او من خلال التصبيغ بواسطة صبغتي الايودين او زيل نيلسون المعدلة والكشف بواسطة المجهر الاعتيادي. اظهر فحص الخضراوات تلوثا فيها بالعديد من الاطوار الطفيلية (البيوض، الاكياس و الاكياس البيضية). وبشكل عام الخضروات من المحلات العامة اظهرت نسبا اعلى للتلوث من الاسواق التجارية. طفيلي *Entamoeba histolytica* كانت الاكثر انتشارا وبتردد21.1% يليها وعلى الترتيب *Giardia lamblia* و *Cryptosporidium parvum* و *Hyminolepis nana* و *Echinococcus granulosus* وبنسب 12.8% ، 5.4 %، 4.2 %، 3.5 % و2.3 % على التوالي لكل طفيلي. الفجل والكرفس والكراث كانوا الأكثر تلوثا بالمقارنة مع الخيار والطماطم والخس و الريحان والملفوف. اعلى نسبة للتلوث كانت في منطقة راس الجسر، نسب عالية أيضا سجلت في مناطق الواسطي وواحد حزيران واقل تلوث كان في منطقة رحيم اوة. استنتجت دراستنا ان الطفيليات كانت شائعة في الخضراوات التي تؤكل بشكل نيء في الاسواق المحلية اكثر من المحال التجارية، الفجل والكرفس والكراث كانوا الأكثر تلوثا بالطفيليات متبوعة بالخيار والطماطم والخس والريحان والملفوف. الاطوار الكيسية للطفيليات *Entamoeba histolytica* و *Giardia lamblia* كانت أكثر الطفيليات المعزولة شيوعا. لوحظ وجود تلوث بأصناف متعددة من الطفيليات في كل أنواع الخضراوات المفحوصة.