Al-Mozan & Dakhil *J Pure Appl Microbiol,* **13**(**2**), 1103-1110 | June 2019
Article 5553 | https://dx.doi.org/10.22207/JPAM.13.2.49

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

# Prevalence of Parasites in Fresh Vegetables from Two Regions of Thi-Qar Province, Iraq

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## Abstract

**To detect parasitic contamination of fresh vegetables and to know the extent of relationship between the prevalence of parasites on vegetables and its infection rate in the hospitals and the extent of vinegar effect (5% Acetic acid) to kill the parasites, (128) samples of six different fresh vegetable types such as Garden Cress, Leek, Radish, Lettuce, Celery and Basil. And (30) additional samples from Leek were collected from local markets of two cities in Thi-Qar province. Sedimentation technique was used for samples examination. *Giardia lamblia* with (71.1 %) was the dominant parasite of protozoa and *Ascaris lumbricoides* (15.6%) was the common parasite of helminthes therefore the significant differences were found by using the statistical analysis (T- test) in level P< 0.05. One hundred and thirteen (113) samples were contaminated with parasites, Leek and Basil with rate (100%) were full contamination with parasites while Lettuce (70%) was the lower. The direct relationship wasn't found between percentage of parasites contamination for fresh vegetables in sale markets and percentage of intestinal parasitic infections in hospitals that near of its. There was relationship between the higher rate of prevalence *G. lamblia* and *E. histolytica* on fresh vegetables and infection of these parasites in the hospitals. No significant differences between rate of infection for male and rate of infection for female by using (T- test) in level P< 0.05. There were some parasites such as *Diphyllobotherium latum*, *Schistosoma japonicum*, *Heterophyes heterophyes* that are rarely or not found in Iraq were isolated from contaminated samples and this indicates to irrigation it by water contaminated with sewage or fertilization it with faces of foreign people that coming to work in Iraq and biodiversity upset and occurrence of new species did not exist in Iraq at the expense of other types. There was positive effect for vinegar (5% Acetic acid) to kill the most parasites except cysts of *G. lamblia* and ova of *E. vermicularis*.**

**Keywords:** Intestinal parasitic, Schistomiasis, Contaminated vegetables, *Diphyllobotherium latum.*

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(Received: 03 March 2019; accepted: 04 May 2019)

**Citation:** Hanaa Daaj Khalaf Al-Mozan and Khalid Majeed Dakhil, Prevalence of Parasites in Fresh Vegetables from Two Regions of Thi-Qar Province, Iraq, *J Pure Appl Microbiol*., 2019; **13**(**2**): 1103-1110. doi: 10.22207/JPAM.13.2.49

## INTRODUCTION

Vegetables are essential for good health, also are a major component of human diet in every family. They are vital energy contributors that are depended by all levels of human as food supplement[1] or useful nutritional factors where human consumes it to renew cells of its body. Its benefit comes from containing it high carbohydrate, vitamins, minerals and fiber contents. According to recommendations of Joint FAO/WHO Expert Consultation, 400g of vegetables and fruits should be eaten to avoiding inveterate disease like heart disease, cancer, diabetes and obesity, in addition to the prohibition and reduction of many micronutrient deficiencies particularly in developing countries[2].

However, vegetables are very risk source for dispersal microbial infections especially parasitic infection[3], where consumption of fresh vegetables plays the major role in transmission of parasitic food-borne illness[4].

Contaminated vegetables contain different parasites comprises many species of both protozoa (*Entamoeba histolytica, Giardia lamblia, Entamoeba coli, Balantidium coli, Isospora belli* and *Cryptospordium spp*.) and helminthes as *Strongyloides stercoralis, Trichuris trichiura, Enterobius vermicularis, Fasciola hepatica, Ascaris lumbricoides, Toxocara spp, Hymenolepis nana, Hymenolepis diminuta* and *Taenia spp*.[5].

Vegetables can be contaminated in many ways like polluted hands of farmers, workers, marketers or buyers; water using for washing product; pending transport or packaging and soil in which the vegetables were planted that polluted by fresh untreated manure used to fertilization it or with dirty water that used to its irrigation[6]. Presence of parasites on fresh fruits and vegetables has been documented from both developed and developing countries[7,8].

For treat, it was concluded from a study has been conducted on fresh vegetables and fruits sold in Lokoja, that salt water using to wash vegetables and fruits is very beneficial to kill parasites[9].

## MATERIALS AND METHODS

### Samples collection

The fresh vegetables samples are (128) including six types in addition to (30) samples of the most serious contamination type (Leek) have been collected from local markets in city of Nassiriyah that were irrigated with water of Euphrates river and river of Tigris and water of well and local markets in city of Suq - AL- Shuyukh that were irrigated with water of Euphrates river where was receiving wastewater that was being thrown on it, also were irrigated with well water

### Preparation of samples[10]

For purpose preparation of vegetable's samples, the coming steps were followed:

The samples were transported from sale's markets to the laboratory and removed the parts that are not eaten.

The included parts in the examination, 100g were weighted and some of vegetables were cut to small parts.

The samples were washed with normal saline (0.9%) on dishes with volume one litter and soaked with suitable amount of water and leaved for 24 hour.

The samples were raised from dish and put on metal sieve for removed the big blotches, the vegetables had rinsed by brush with small amount from special water then the vegetables were raised and put on side.

Washing water was transported (after filtration it on metal sieve) into dish which was left to stand on the bench for one hour for proper sedimentation.

The supernatant was discarded and sediment was examined.

### Soaking in vinegar (5% Acetic Acid)

After both (1) and (2) steps of preparation of samples, the vegetables were washed with water and soaked with vinegar for 15 minute ( this way was done especially for the most difficult and polluted sample (Leek) where 30 additional samples from it were added, then the rest steps have been completed in preparation of samples.

### Concentration method by Sedimentation Procedures

The sediment was transferred into tube with 10 ml normal saline and centrifuged for five minutes at 2500 rpm. The supernatant was decanted while the sediment was resuspended with 10 ml normal saline. This was centrifuged, the supernatant was decanted, later the sediment was transferred to a clean glass slide. A drop of iodine was added in order to staining the cysts,

then it was covered with a cover slip avoiding air bubbles and over floating. Finally, it was examined by microscope[10].

**General Hospitals**

After taking patient's consent to include him within samples of study under supervision of health staff in the hospitals, its stool sample was taken in clean container and some information about him such as name, age and sex were recorded.

**Direct stool examination**

Stool samples were examined with naked eye for color, odor, and presence of blood or mucous. In some instance, parasites may be seen on gross inspection as in the case of round worm, pin worm or tape worm proglottides[11,12]. Then they were examined Microscopically by direct method with using normal saline and logal, s iodine[13,14].

**The statistical analysis**

The statistical analysis was performed in this study by using ANOVA test and T-test according to[15].

**Table 1.** Distribution of detected parasites in the examined fresh vegetables

| Type of parasite | N. of contaminated samples | Percentage |
|---|---|---|
| *Giardia lamblia* | 91 | 71.1 % |
| *Entamoeba histolytica* | 26 | 20.3 % |
| *Balantidium coli* | 29 | 22.7 % |
| *Trichomonas hominis* | 50 | 39.1 % |
| *Entamoeba coli* | 6 | 4.7 % |
| Coccidia | 5 | 3.9 % |
| *Enterobius vermicularis* | 13 | 10.2 % |
| *Heterophyes heterophyes* | 1 | 0.8 % |
| *Hymenolepis nana* | 1 | 0.8 % |
| *Strongyloides stercoralis* | 12 | 9.4 % |
| *Taenia saginata* | 1 | 0.8 % |
| *Diphyllobotherium latum* | 2 | 1.6 % |
| *Ascaris lumbricoides* | 20 | 15.6 % |
| *Ancylostoma duodenale* | 13 | 10.2 % |
| *Trichuris trichiura* | 5 | 3.9 % |
| *Schistosoma mansoni* | 1 | 0.8 % |
| *Schistosoma japonicum* | 1 | 0.8 % |
| *Schistosoma haematobium* | 2 | 1.6 % |
| *Fasciola hepatica* | 1 | 0.8 % |
| *Dipylidium caninum* | 1 | 0.8 % |
| Larva of nematoda | 3 | 2.3 % |

$T_{Calculated}$ = 2.855 , d.f = 20 , Sig. = 0.01 , $T_{Tabulated}$ = 1.725

**RESULTS**

Protozoa have higher percent than helminthes where *G. lamblia* was outstanding of protozoa with rate (71.1%) while prevailing of helminthes was ova of *A. lumbricoides* with percent (15.6%) and presence of several rare parasites such as *D. latum* with rate (1.6%) and *H. heterophyes*, *S. mansoni*, *S. japonicum*, and *D. caninum* with percent (0.8%) for each one of them so the differences were significant (Table 1).

Out of 128 samples of the six types of fresh vegetables, 113 were contaminated by parasites with rate (88.3%). Leek and Basil appeared with rate (100%) while Lettuce with the lowest percent of contamination (70%), therefore the differences were significant (Table 2).

About infection rate with intestinal parasitic in hospitals and contamination percent of vegetables in closed markets of these hospitals, the highest percentage has been recorded in two hospitals of Nassiriyah city was 46.7% while the contamination of their markets was 85.9%. And infection rate with intestinal parasitic in Suq- AL-Shuyukh general hospital was 41.8% comparison with contamination of Suq-AL-Shuyukh markets that was 90.6% (Fig. 1) .

As for patients with intestinal parasites were visiting Suq-AL-Shuyukh general hospital, infection rates of male and females were (56.5%) and (43.5%) respectably, so the difference doesn't significant. *E. histolytica* with percent (92.9%) is considered the highest percentage. A higher percentage of infection (24.9%) in males and (16%) in females appeared in (1-10) age group while

**Table 2.** Distribution of parasitic contamination rate on six type of fresh vegetables samples

| Type of vegetables | N. of examined samples | N. of contaminated samples | Percentage |
|---|---|---|---|
| **C**elery | 24 | 21 | 87.5% |
| Leek | 24 | 24 | 100% |
| Garden Cress | 24 | 23 | 95.8% |
| Radish | 24 | 19 | 79.2% |
| Lettuce | 20 | 14 | 70% |
| Basil | 12 | 12 | 100% |
| Total | 128 | 113 | 88.3% |

$T_{Calculated}$ = 17.784, d.f = 5, Sig. = 0.00, $T_{Tabulated}$ = 2.015

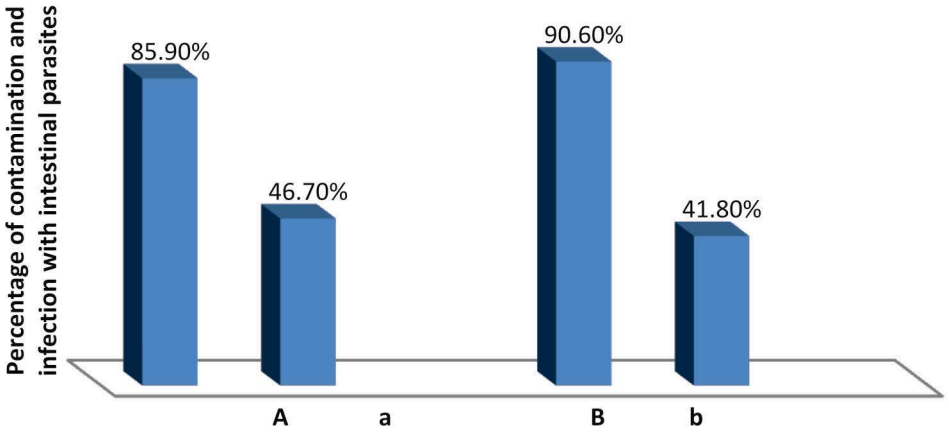



**Fig. 1.** Percentage of infection with intestinal parasites in hospitals comparison with closed markets of its. A: The markets of AL- Nassiriyah; a: Nassiriyah Maternity and Children hospital and Nassiriyah general hospital; B: The markets of Suq – AL- Shuyukh; b: Suq-AL-Shuyukh general hospital;
**Note:** Nassiriyah Maternity and Children hospital was receive children aged fourteen years old and less than that age while Nassiriyah general hospital was receive persons aged bigger than fourteen years old.

the lowest percentage (0.4%) has been found at age groups (51-60), (61-70) and (71-80) years old (Table 3).

In connection with infected persons with intestinal parasites in Nassiriyah Maternity and Children hospital and Nassiriyah general hospital, rates of infection males and female were equal with rate (50%). *E. histolytica* with (85.5%) was the dominant. The highest percent of infection was (37.2%) in males and (41.3%) in females at (1-10) age group while the lowest percentage (1.2%) for male and (0.0%) for female has been found at age group (61-70) (Table 4).

Pollution rate with different phases of parasites in Leek samples that were soaked in vinegar was (30%) comparison with samples of Leek that washing with water very good (70%) and samples of Leek without washing (100%). As for parasites were found in samples of Leek without washing were: cysts and trophozoites of *G. lamblia*, Oocysts of Coccidia, larva of Nematoda, *B. coli*, *T. hominis*, ova of *A. lumbricoides*, *E. coli*, ova of *E.*

**Table 3.** Percentage of infection with intestinal parasites according to age groups and six factor in Suq-AL-Shuyukh general hospital

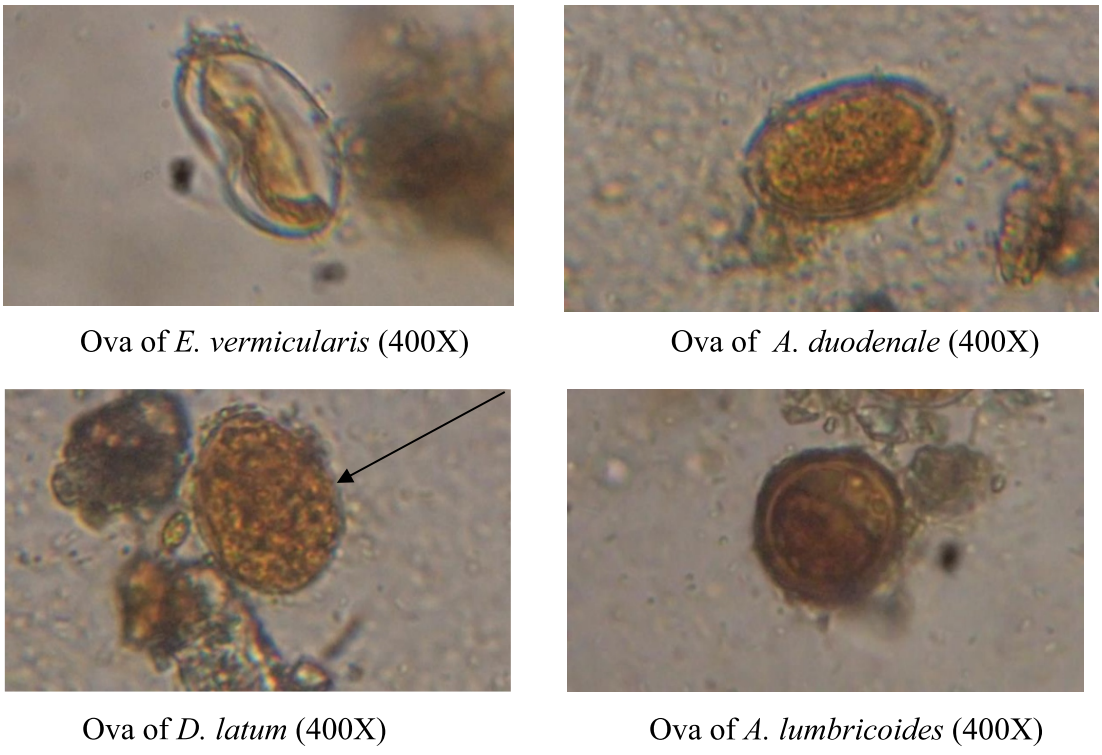
| Age group | Sex | | Type of parasite | | |
|---|---|---|---|---|---|
| | Male | Female | *E. histolytica* | *G. lamblia* | *E. histolytica* + *G. lamblia* |
| (1-10) | 67 (24.9%) | 43 (16%) | 107 (39.8%) | 3 (1.1%) | 0 (0.0%) |
| (11-20) | 1 (0.4%) | 7 (2.6%) | 7 (2.6%) | 1 (0.4%) | 0 (0.0%) |
| (21-30) | 34 (12.6%) | 27 (10%) | 56 (20.8%) | 4 (1.5%) | 1 (0.4%) |
| (31-40) | 40 (14.9%) | 35 (13%) | 65 (24.2%) | 4 (1.5%) | 6 (2.2%) |
| (41-50) | 7 (2.6%) | 2 (0.7%) | 9 (3.3%) | 0 (0.0%) | 0 (0.0%) |
| (51-60) | 1 (0.4%) | 1 (0.4%) | 2 (0.7%) | 0 (0.0%) | 0 (0.0%) |
| (61-70) | 1 (0.4%) | 1 (0.4%) | 2 (0.7%) | 0 (0.0%) | 0 (0.0%) |
| (71-80) | 1 (0.4%) | 1 (0.4%) | 2 (0.7%) | 0 (0.0%) | 0 (0.0%) |
| Total | 152 (56.5%) | 117 (43.5%) | 250 (92.9%) | 12 (4.5%) | 7 (2.6%) |

The statistical analysis $T_{Calculated}$ =1.386, d.f =7, Sig= 0.2, $T_{Tabulated}$ = 1.895LSD (0.05) = 29.75, Sig = 0.02, $F_{Calculated}$ = 4.523

**Table 4.** Percentage of infection with intestinal parasites according to age groups and six factor in Nassiriyah Maternity and Children hospital and Nassiriyah general hospital.

| Age group | Sex | | Type of parasite | | |
|---|---|---|---|---|---|
| | Male | Female | *E. histolytica* | *G. lamblia* | *E. histolytica + G. lamblia* |
| (1-10) | 64 (37.2%) | 71 (41.3%) | 119 (69.2%) | 9 (5.2%) | 7 (4.1%) |
| (11-20) | 7 (4.1%) | 2 (1.2%) | 7 (4.1%) | 1 (0.6%) | 1 (0.6%) |
| (21-30) | 4 (2.3%) | 5 (2.9%) | 8 (4.7%) | 1 (0.6%) | 0 (0.0%) |
| (31-40) | 3 (1.7%) | 4 (2.3%) | 5 (2.9%) | 2 (1.2%) | 0 (0.0%) |
| (41-50) | 4 (2.3%) | 2 (1.2%) | 4 (2.3%) | 2 (1.2%) | 0 (0.0%) |
| (51-60) | 2 (1.2%) | 2 (1.2%) | 3 (1.7%) | 1 (0.6%) | 0 (0.0%) |
| (61-70) | 2 (1.2%) | 2 (1.2%) | 1 (0.6%) | 1 (0.6%) | 0 (0.0%) |
| Total | 86 (50%) | 86 (50%) | 147 (85.5%) | 17 (9.9%) | 8 (4.7%) |

The statistical analysis $T_{Calculated}$ =.000, d.f =6, Sig= 1, $T_{Tabulated}$ = 1.943LSD (0.05) = 9, Sig = 0.000, $F_{Calculated}$ = 18151



Ova of *E. vermicularis* (400X) Ova of *A. duodenale* (400X)

Ova of *D. latum* (400X) Ova of *A. lumbricoides* (400X)

**Fig. 2.** Ova of some parasites that identified in the present study

*vermicularis* and *S. stercoralis*, while the parasites were found in samples that were proved its contamination from samples of Leek that soaked in vinegar were only cyst of *G. lamblia* and ova of *E. vermicularis* (Table 5).

**Table 5.** Effect of vinegar (5% Acetic Acid ) on percentage of contamination with intestinal parasites that diagnosed on vegetables (*Allium porrum* (Leek) as example).

| Method of washing samples of vegetables | N. of examined Leek | N. of contaminated Leek | Percentage |
|---|---|---|---|
| Without washing | 10 | 10 | 100 % |
| Washing with water | 10 | 7 | 70 % |
| Soaking in vinegar (5% Acetic Acid) after Washing with water | 10 | 3 | 30 % |

**DISCUSSION**

Freshly eaten vegetables such as Apium graveolens, Lactuca sativa are common sources of parasitic prevalence, and there are a lot of attention on this problem because people eat vegetables increasingly, thus number of infected persons with parasites will be in progress[16].

A study on parasitic contamination of consumed fresh vegetables in Tabriz, Iran showed that (40%) of markets vegetables and (76%) of gardens vegetables were contaminated with different parasites such as *Giardia* cysts (7%), *Fasciola* egg (3%) and *Ascaris* egg (1%)[17].

Protozoa rate were higher than percent of helminthes, *G. lamblia* with (71.1%) represented the most common parasite which is in agreement with[18]. The reason belong to *G. lamblia and E. histolytica* are the most common intestinal parasites in the world and transmission of them directly (without need intermediate host)[19].

*A. lumbricoides* with (15.6%) was dominant which is in agreement with[18,20,21,22]. The reason due to presence thick cortex around ova of *A. lumbricoides* that give it high resistance against hard environmental[23], and to presence mass of faece around ova of *A. lumbricoides* stay on it for additional period of time, as well as most of parasitic infections that transport via soil are *A. lumbricoides*, *T. trichiura* and Hook worm[24].

Presence of helminthes such as *Schistosoma mansoni* (0.8%), *Schistosoma japonicum*(0.8%), *Dipylidium caninum* (0.8%) and *Diphyllobotherium latum* (1.6%) that should be rarely found or didn't found in Iraq and its presence may be result in using of feces of foreign humans in order to fertilization of lands and sudden changes in water and air components that have prevailed in Iraq and emergence of species and extinction of other organism. This is in agreement with[25] who record many uncommon parasites such as *S. mansoni*. Also is in agreement with[26,27] who isolated several rarely or uncommon parasites such as *D. latum* and *S. haematobium* respectively

Parasitic contamination rate of fresh vegetables was (88.3%) which is in agreement with[18] and disagreement with[28,29,30]. Differences and similarity in the results belong to difference of pesticides using among varied regions[18]. Also this may be due to water that using for irrigation these vegetables where its resource was river of Euphrates which was receiving wastewater that was thrown in it.

The differences among contamination rates of the six types of fresh vegetables were significant which is in agreement with[18], Leek with rate (100%) was acquired full percentage which is disagreement with[18] where Garden Cress was the highest contamination in her study with rate (93.5%). As for Lettuce was the lowest contamination with rate (70%) which is in agreement with[31]. The reason of full contamination for Leek might be return to coiling its leaves that became good refuges for parasites and protected these parasites from bad environmental conditions, the lowest contamination of Lettuce may be to irrigation it by water of river or water of well at present instead of irrigation it with waste water that contains faeces of human which was

done in the previous periods.

Direct relationship wasn't found between percentage of contaminated fresh vegetables with parasites in sale markets and percentage of intestinal parasitic infections in the hospitals that lie near to it.

There was relationship between the higher rate of *G. lamblia* and *E. histolytica* on fresh vegetables and their infection in the hospitals, although infection rate of *E. histolytica* upper than infection rate with *G. lamblia* but contamination rate of fresh vegetables with *E. histolytica* was lower than rate of contamination fresh vegetables with *G. lamblia* on the same fresh vegetables. The reason might be return to presence of *G. lamblia* on vegetables with trophozoite phase more than its presence with cyst phase while *E. histolytica* was found in these vegetables with cyst phase more than trophozoite of it. Thus trophozoite was less resistance for very hard conditions comparison with cyst. Fortunately, the most of parasites that found on fresh vegetables need to intermediate host in order to become infective to human so they didn't record any percentage in the hospitals.

From actuality, that cyst of *E. histolytica* can be survive in soil for eight day which is make cyst of *E. histolytica* similar to ova of helminthes with its ability on transmitting via contaminated soil, and that soaking of fresh vegetables with (5% Acetic acid) lead to killing cyst of *E. histolytica* without effecting on freshness of vegetables[32], Leek was chosen from included fresh vegetables in this study in order to soaking it with vinegar as its full contamination with parasites .

## CONCLUSION

Wastewater was throwing in river of Euphrates played the main role of increasing parasitic contamination of fresh vegetables. The presence of genera or species of parasites that is not common in Iraq or originally not found in Iraq resulting from disruption of biological diversity that prevailed in Iraq, animal fertilizers using (animal feces and feces of humans coming from foreign countries) in fertilization of agricultural land. Vinegar (5% acetic acid) was the best treatment in order to kill most of parasites except cysts of *G. lamblia* and ova of *Enterobius vermicularis*.

## ACKNOWLEDGEMENTS

We would like to express our thanks to bacteriologi Abdul Mohsin Gameel Mankhi in Nassiriyah Maternity and Children hospital, laboratory technician Abdul Mahdi Hashim Naser in Suq- AL-Shuyukh general hospital and an engineer Ikhlas Daaj Khalaf in Suq- AL-Shuyukh PHc District for all search processing.

## CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

## FUNDING

None.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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