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Immunological and Molecular Investigation of the Level of Parasite Contamination of Some Vegetables Sold in the Local Markets of Ramadi City – Iraq

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Abstract

Some vegetables were randomly collected from the local markets of Ramadi city - Anbar province – Iraq .And at 250 gm for each species for a period of 10 months (from 1/2/2019 - 1/12/2019), the vegetables included:
Foeniculumvulgarae ، *Beta vulgaris* ، *Lactuca sativa* ، *Petroseliniumcrispum* ، *Lepidium* sativum ،*Brassica oleracea* ، *Brassica rapa* ، *Lycopersionesculentum* ، *Solanumtuberosum* ، *raphanussativus* ، *Allium ampeloprasum* ، *Apiumgraveolens*.
After washing with distilled water, protozoa and parasitic worms were detected, and several methods were used to detect them, such as the sedimentation method, floatation , pigmentation and cassette method, as well as molecular detection method of the *Cyclospora* parasite. The results showed that it is *Lactuca sativa*were most polluted plants by parasites , Followed by *Feniculumvulgarae* . And by percentage reached(21.45631 and 20.57971%) respectively , while the least polluted plants with parasites were *Petroselinium crispum* and *Solanum tuberosum* (9.699321, 9.796314%), respectively.
The results also showed that the most prevalent parasitic protozoa species was *Giardia lambelia* cyst, with a percentage of 21.94915% and with significant differences from the rest of the other parasitic species at the probability level P≥0.05,
It is followed by *Entamoeba histolytica* cyst with a percentage of 21.44068 % .
Whereas, the least common types of parasitic protozoa were *Cyclospora catenensis* Oocyst , A percentage of 6.779661% followed*Cryptosporidium parvum* Oocystand *Isospora* spp. Oocyst ,A percentage of 7.627119% and 7.71186 % respectively .
As for helminths , they were the most common species*Fasciola hepatica* eggswith a percentage of 19.236% , with significant differences from other parasitic species at the probability level P≥0.05 ,Followed by *Trichuris trichura* eggs, with a percentage of 15.00682% . Whereas, the least common parasitic worms were *Taeneaspp*. Eggs with a percentage of 3.819918% followed by *Strongyloides stercoralis* eggs with a percentage of 9.41337% .
Keyword:contamination, *Lactuca sativa, Cyclosporacatenensis,* Oocyst, vegetables.

Introduction

Vegetables are an essential part of a healthy person's diet due to their nutritional value. Raw vegetables are a great source of energy because they contain vitamins (C, E, A, and B_{12}), dietary fiber and mineral elements representedby iron, calcium, sodium, potassium, iodine, carotenoids, niacin and riboflavin, and are characterized by low calories and not containing cholesterol, and

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most of them protect against obesity diseases, and are described as being a self-contained mobile pharmacy, where regular consumption is associated with a lower risk of disease the heart, blood vessels, stroke, and some types of cancer are also anti-oxidants. Green vegetables are also considered one of the most important appetizers and food additives that accompany human diets[1],[2].

The cultivation of vegetables expanded all over the world in a striking way as well as the use of chemical fertilizers , However, on popular farms, the use of animal fertilizers (animal droppings) has been and is still common (especially in the present study area of Ramadi - Iraq) , Therefore, parasitic, bacterial and viral infections increased through the frequent use of untreated animal manure as fertilizer, which serves as a source of enhancement for zoonotic parasitic infections , Unhealthy consumption of vegetables (eating raw and unwashed vegetables in a proper way) and using untreated wastewater properly , And the supply of water contaminated with wastewater for irrigation, post-harvest treatment, as well as during collection and transport to the places of sale and the way to prepare them for food, whether in homes or restaurants, is an important factor in the occurrence of various parasitic infections [3],[4] .

Parasites are widespread in all countries of the world, including developed countries, with an estimated infection rate of 60%, and their likelihood of being found in societies where health awareness is low , A person is infected with these parasites through contaminated water, meat, or contaminated fruits and vegetables. Some of them are transmitted through arthropods, and some are transmitted from the mother to the fetus.The human being is also a host to more than 100 different types of parasites that may be round or striped worms, flukes or protozoa , as they affect human tissues and organs causing various symptoms such as diarrhea, bloating, lack of absorption of nutrients, vasculature, dysentery and anemia, some of which lead to asthma or arthritis in addition To skin ulcers [5].

The current study was designed to determine the contaminated parasites of some types of vegetables available in the local markets of the city of Ramadi - Iraq, which people eat continuously and consider it a daily basic food .

Methods

Some vegetables were randomly collected from the local markets of Ramadi city - Anbar province – Iraq .And at 250 gm for each species for a period of 10 months (from 1/2/2019 - 1/12/2019), the vegetables included:

Foeniculumvulgarae ، *Beta vulgaris* ، *Lactuca sativa* ، *Petroseliniumcrispum* ، *Lepidium* sativum ،*Brassica oleracea* ، *Brassica rapa* ، *Lycopersionesculentum* ، *Solanumtuberosum* ، *raphanussativus* ، *Allium ampeloprasum* ، *Apiumgraveolens*.

Samples were placed in nylon sac separately and labeled and transferred to the laboratory to search for cysts or oocysts phases of protozoa and egg worms within six hours of collecting the sample ,Vegetables washed with a distilled water separately for each type and soaked in water for one whole night. The washed and soaked water was filtered through a piece of medical gauze to remove large and coarse substances,Then the filtrate was taken and placed in test tubes of 50 ml capacity and placed in the centrifuge for 5 minutes at a speed of 3000 rpm for precipitation , Then the filtrate was poured, a portion of the precipitate was taken and placed on a glass slide and examined under the power of 40X to investigate the Cysts/Oocysts of protozoa and the helminthes

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eggs ,Where 5 slides of each sample were examined to confirm the infection or not and used floatation method with a shethers solution (6.5 g of phenol mixed with 500 g of sugar and dissolved with 350 ml distilled water, and upon completion of the dissolution the volume was completed to a liter) , For each sample, the following tests were performed:

1 - 5 replicates by precipitation method.
2- 5 replicates using the shethers solution for floating .
3- 5 replicates by adding iodine stain , whether it is for floating or sedimentation
4 - 5 replicates using the modified ziehl neelsen stain to reveal some of protozoa cysts/oocysts .
5- The immune cassette technology (Antigen + Antibody) was used for three protozoa parasitic types: *Cryptosporidium*, *Entamoeba*, *Giardia*.
6- Molecular detection of *Cyclospora* samples, using the nPCR method and using the primers :

SAD For 5-G CAGTCACAGGAGGCATATATCC-3
SAD Rev 5-ATGAGAGACC TCACAGCCAAAC-3

After concentrating the samples of Oocysts and extracting the DNA from the samples.
The data was analyzed using a Chi square (X^2) test to examine the relationship between parasites associated with vegetables and their incidence with these parasites.

Results and Discussion

During the period between 1/2/2019 until 1/12/2019, twelve types of vegetables were collected (Picture 1),Through laboratory examination, it was found that they were contaminated with some types of worm eggs, including both eggs *Ascarislumbricoid* ،*Fasciola hepatica* و *Hymenolepis nana* ،*Trichuristrichura* ،*Toxocara spp.* ،*Ancylostomaduodenal* ،*Strongyloidesstercoralis, Teanea spp.* ,and the parasitic protozoa were *Entamoebahistolytica* cyst ، *Entamoeba coli*cyst ، *Giardia lambelia* cyst ، *Cryptosporidium parvum*Oocyst ، *Blastocystishomenos* cyst ، *Isosporaspp*.Oocyst،*Balantidium coli* cyst ,*Cyclosporacatenensis*Oocyst .
Table (1) shows the type of vegetables examined and the percentage of pollution by parasites, as well as the amount of pollution obtained for each type of vegetables , The results showed that it is *Lactuca sativa* It was the most polluted plant, followed by*Foeniculumvulgarae* and by percentages (21.45631, 20.57971%), respectively , While it was the least polluted plants *Petroseliniumcrispum* and *Solanumtuberosum* ,(9.699321, 9.796314%), respectively.
The results of the [6]study in Al-Diwaniyah city, Iraq, which included 9 varieties of vegetables (*Foeniculumvulgarae*, *Beta vulgaris*, *Lactuca*, *Petroseliniumcrispum*,*Lepidium*, *Brassica oleracea*, *Brassica rapa*, *Lycopersionesculentum*, *Solanumtuberosum*).And it proved that the *Lactuca sativa* plant is the most polluted vegetable with parasites, and the percentage of pollution reached 26% ,This may be due to the fact that vegetables are more in contact with the soil surface, in addition to the abundance of leafy folds in *Lactuca* , which carries many pollutants, which may be eggs of worms or cysts/oocysts of parasites for parasitic protozoa , in addition to the quality of the water used and the appropriate climate, As for the study of [7]For commercial markets in Brazil, it was clarified that the *Lactuca sativa* had 89% contamination after taking 200 samples (100 from *Lactuca sativa* and 100 from *Coriandrumsativum*), and this is consistent with our current study.
While the [8]study in Jordan recorded a rate of tomato contamination with parasitic species more than vegetables and was estimated at 24%, the reason for the emergence of this ratio was that the

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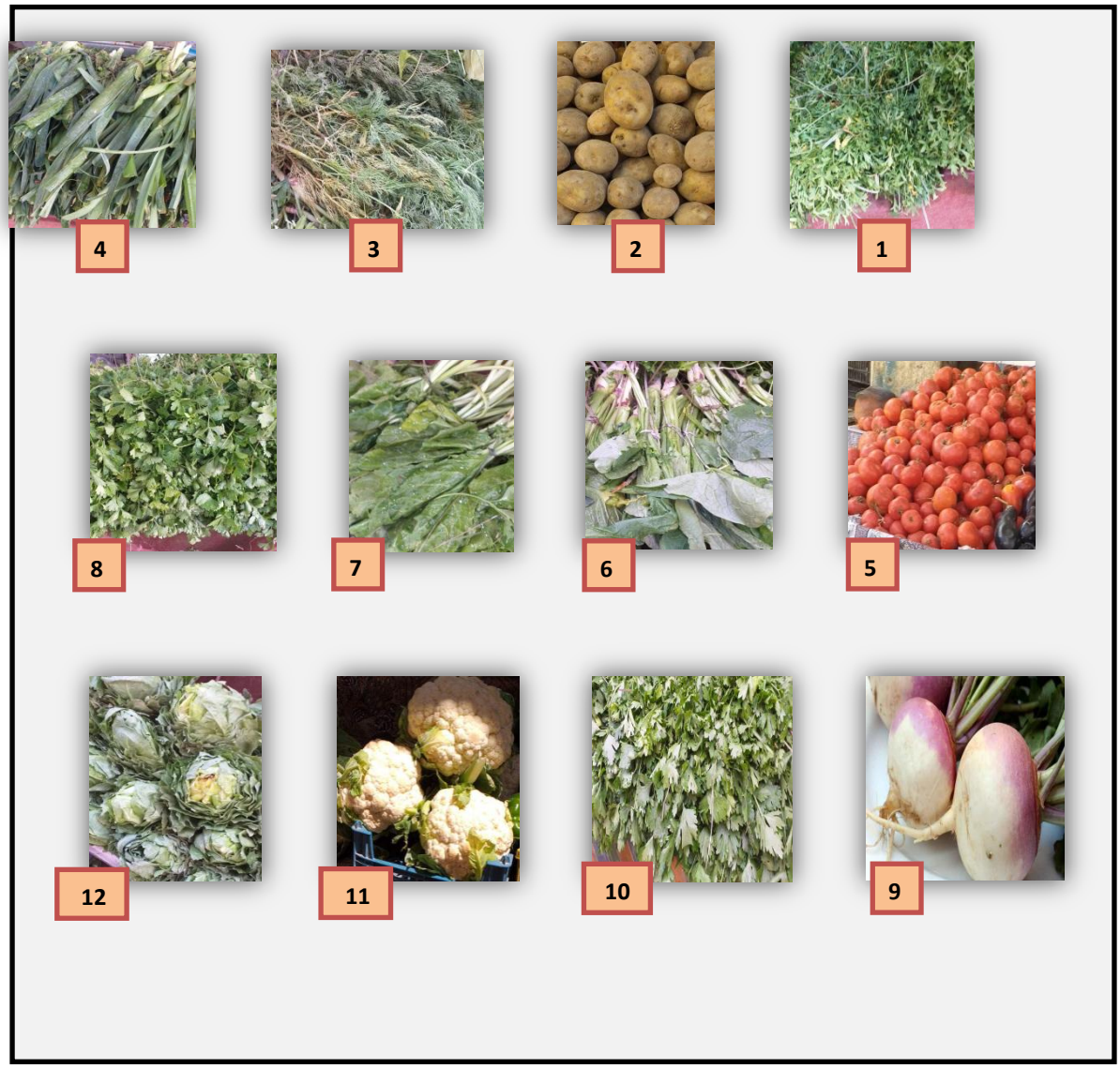
collection of plants was from the local market and not from the farm before it was cleaned by the workers, and indicated that it collected them from the farm directly will increase the rates of contamination by parasites. This is contrary to our studies.



Picture (1) showing the studied plants

1. *Lepidium sativum* **. 2.***Solanumtuberosum* **. 3.** *Foeniculum vulgarae***. 4.** *Allium ampeloprasum* **.**
5.*Lycopersionesculentum* **. 6.***Raphanussativus* **. 7.** *Beta vulgaris* **. 8.***Apiumgraveolens* **.**
9. *Brassica rapa* **. 10.***Petroseliniumcrispum* **. 11.** *Brassica oleracea* **. 12.***Lactuca sativa* **.**

Table (1) shows plants type examined and the percentage of contamination with parasites

NS	NE	NC	PC %
Foeniculumvulgarae	1035	213	20.57971
Apiumgraveolens	1031	201	19.49564
Allium ampeloprasum	1034	200	19.34236
Raphanussativus	1032	124	12.0155
Solanumtuberosum	1031	101	9.796314
Lactuca sativa	1030	221	21.45631

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Brassica rapa	1030	189	18.34951
Brassica oleracea	1036	167	16.11969
Lepidium sativum	1034	123	11.89555
Petroseliniumcrispum	1031	100	9.699321
Lycopersionesculentum	1030	118	11.45631
Beta vulgaris	1035	156	15.07246
Total	**12389**	**1913**	**15.44112**

NS= Name of vegetable types screened **, NE=**Number of vegetable types Examined **, NC=** Number contaminated **, PC=** Percentage contamination **.**

Table (2) showed that the most prevalent species of parasitic protozoa were *Giardia lambelia* cyst, with a percentage of 21.94915% and with significant differences from the rest of the other parasitic species at the probability level P≥0.05, followed by *Entamoebahistolytica* cyst and with a percentage of 21.44068% . The lowest prevalent parasitic species were *Cyclosporacatenensis*Oocyst, with a percentage of 6.779661% , followed by *Cryptosporidium parvum*Oocyst and *Isospora* spp. Oocyst and percentages of 7.627119% and 7.71186% respectively. Picture (2).

As for Table (3), it showed that the most prevalent parasitic worms were *Fasciola hepatica* eggs, with a percentage of 19.236% , with significant differences from the rest of the other parasitic species at the probability level of P≥0.05 , followed by *Trichuristrichura* eggs , with a percentage of 15.00682% . Whereas, the least common parasitic worms were *Taenea* spp. Eggs with a percentage of 3.819918% followed by *Strongyloidesstercoralis* eggs with a percentage of 9.41337% . Picture (3) .

After examining a group of fruits and vegetables, [9]found that Strawberies were the most affected plant, and *Enterobiusvermaculais* eggs were the most common eggs of worms, with a percentage of 18%, while the most common types of Cysts for parasitic protozoa were *Giardia lambelia*, with a percentage of 9% With significant differences at the probability of p≥0.05 ,The reason was explained as the result of improper handling of samples or irrigation water , Irrigation water may come into contact with soil containing many pathogens , Moreover, these pathogens may be viable under distinct conditions of temperature and relative humidity,In addition to that these parasites are in great contact with vegetables and fruits, and this is what poses a great danger to human consumers and their pets , As [10]explained after taking a group of vegetables and fruits from shops in Bauchi - Nigeria, the most common parasitic worms were *Ascarislumbricoides* followed by *Ancylostomaduodenale*, Whereas the most popular protozoa were *Giardia lambelia*, followed by *Entamoebahistolytica* , In a study by [11]When collecting varieties of vegetables, the *Lactuca* was the most polluted plant with parasites, with a percentage of 45.5% , After sedimenting and floating it in the vegetables washing water and staining some of them with the modified Ziehl Nelson stain and others with iodine,It was found that the most present parasitic eggs were *Enterobiusvermicularis* (4.9%), followed by *Hymenolepis nana* (2.8%),Whereas, the most present parasitic protozoa was *Giardia lamblia* cysts (8.8%), followed by *Entamoeba* spp. cysts (6.8%), and this is partly consistent with the current research results , as the result is contrary to our results with regard to the spread and quality of worms eggs , and they were identical to our results in terms of prevalence and contamination of vegetables with parasitic protozoa .

Whereas, the current search results were contrary to the results of both [12]and [13]where the first,

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after examining 260 samples of fresh vegetables, showed that *Entamoebahistolytica* / *dispar* was the most prevalent protozoa , followed by *Entamoeba coli* and *Giardia lamblia* . As for helminths, *Ascarislumbricoides* were the most polluted vegetables, followed by *Strongyloidesstercoralis* and *T. trichiura* , The reason has shown that it is due to the quality of cultivated soil and the quality of water used for irrigation, which would increase the spread of parasitic species.The second researcher showed that *Ascarislumbricoides* (42.2%) were more present in vegetables, and that the prevalence rates of parasitic protozoa were as follows *Entamoebahistolytica* (17.8%), *Entamoeba coli* (15.5%), cyst of *Balantidium coli* (11.1%), and attributed the reason for the incorrect use of irrigation water is the study of [7]for commercial markets in Brazil, he showed that the most common protozoa parasite in *Lactuca*was *Endolimax nana*, followed by *Blastocystishominis*, while helminths were Hookworms and *Trichuristrichiura*.

Some protozoa parasites were diagnosed using a cassette equipped by a company Triage Micro Parasite Panel; *Cryptosporidium* / *Giardia* / *Entamoeba*Combi Test is an immunochromatographic rapid assay, picture (4)

Table (2) shows contamination of vegetables with protozoa

Vegetables	Parasitic Protozoa								Percentage
	Ciliates	flagellates	sarcodina			sporozoa			
	Balantidium coli cyst	*Giardia lambelia* cyst	*Blastocystish omenos* cyst	*Entamoebahist olytica* cyst	*Entamoeba coli* cyst	*Isospora* spp.Oocyst	*Cryptosporidium parvum*Oocyst	*Cyclosporac atenensis*Oo cyst	
Foeniculumvulgarae	18	25	8	24	14	9	10	6	**9.661017***
Apiumgraveolens	15	23	7	24	13	9	10	9	**9.322034**
Allium ampeloprasum	15	23	5	21	20	8	9	9	**9.322034**
Raphanussativus	13	20	3	18	13	7	5	7	**7.288136**
Solanumtuberosum	10	19	2	17	13	6	4	5	**6.440678**
Lactuca sativa	18	26	9	25	14	9	9	4	**9.661017***
Brassica rapa	15	22	8	23	14	9	9	8	**9.152542**
Brassica oleracea	14	21	6	22	14	8	8	5	**8.305085**
Lepidium sativum	10	19	5	18	12	7	7	8	**7.288136**
Petroseliniumcrispum	12	20	6	20	11	6	6	6	**7.372881**
Lycopersionesculentum	12	19	8	20	14	6	6	6	**7.711864**
Beta vulgaris	15	22	8	21	13	7	7	7	**8.474576**
Percentage	**14.15254**	**21.94915***	**6.355932**	**21.44068***	**13.98305**	**7.71186**	**7.627119**	**6.779661**	**100**

*** Indicate significant differences at 0.05 probability level**

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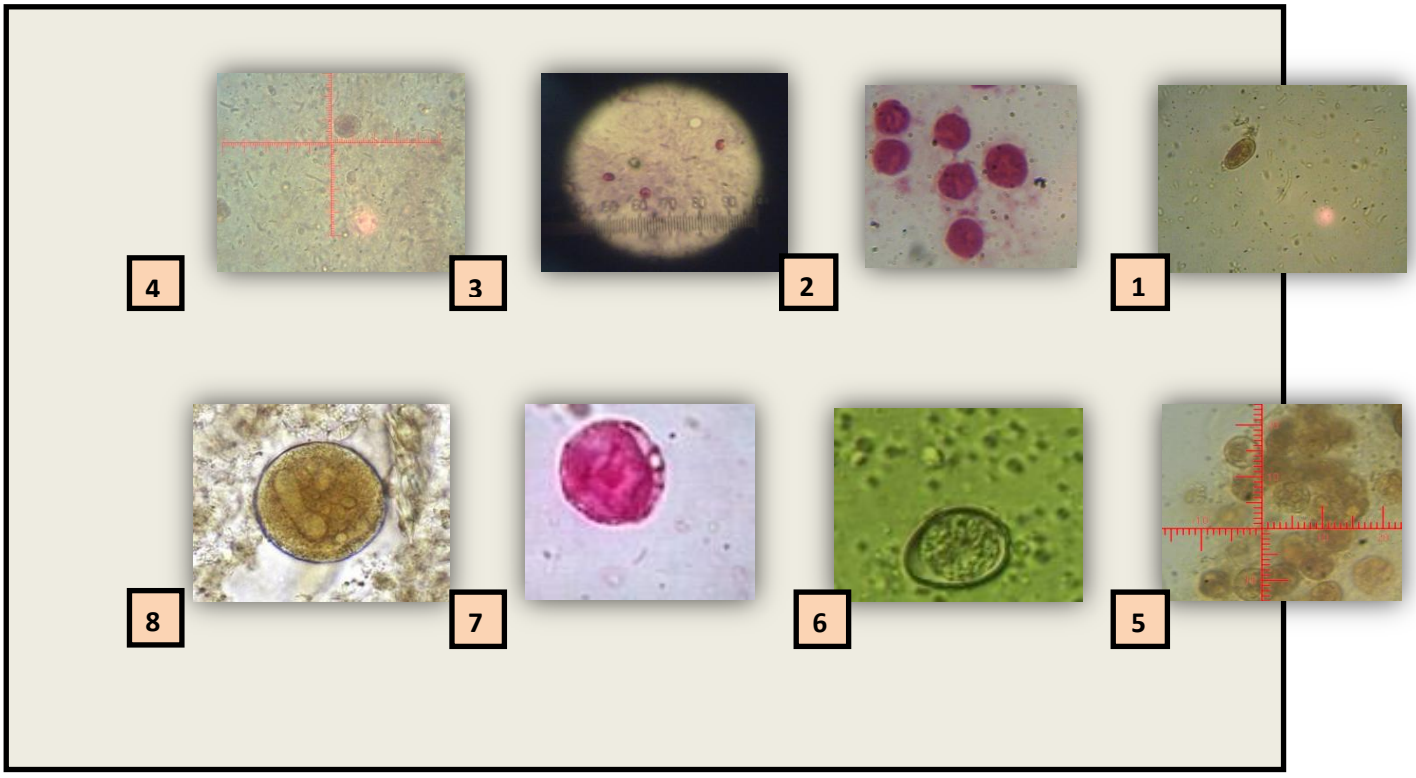

Picture (2) shows the parasitic protozoa under study

1-*Entamoeba histolytica* cyst. **2**-*Cryptosporidium parvum* Oocyst. **3**-*Cyclospora catenensis* Oocyst. **4**-*Giardia lambelia* cyst. **5**-*Blastocystis homenos* cyst. **6**-*Balantidium coli* cyst. **7**-*Entamoeba coli* cyst. **8**-*Isosporaspp*. Oocyst.

explained [14]Triage Micro Parasite Panel examination is one of the accurate methods of diagnosis. 500 samples were examined and only one showed a false positive result compared to the usual methods that showed 3 false positive samples , also [15]explained that the use of the cassettes in the parasite examination are successful and considered to be of high efficiency and appropriate diagnostic accuracy.

Table (3) shows contamination of vegetables with Helminths

Vegetables	Parasitic Helminthes								Percentage
	Platyhelminthes			Aschelminthes					
	Fasciola hepatica eggs	*Hymenolepis nana eggs*	*Taeneasp p. eggs*	*Strongyloides stercoralis eggs*	*Ancylostoma duadenal eggs*	*Trichuris trichura eggs*	*Ascarislu mbricoid eggs*	*Toxocara spp. eggs*	
Foeniculumvulgarae	15	13	4	9	17	18	12	11	**13.50614**
Apiumgraveolens	13	8	4	7	14	16	18	11	**12.41473**
Allium ampeloprasum	15	8	2	8	14	15	18	10	**12.27831**
Raphanussativus	11	4	1	4	4	4	6	4	**5.184175**
Solanumtuberosum	8	4	1	2	1	1	6	2	**3.410641**
Lactuca sativa	16	14	6	12	18	18	12	11	**14.5975***
Brassica rapa	14	13	2	8	12	13	10	9	**11.05048**
Brassica oleracea	13	12	4	5	9	11	9	6	**9.41337**
Lepidium sativum	8	7	2	4	5	4	4	3	**5.047749**
Petroseliniumcrispum	5	2	1	1	0	0	2	2	**1.773533**
Lycopersionesculentum	10	3	0	3	3	2	4	2	**3.683492**
Beta vulgaris	13	8	1	6	8	8	6	6	**7.639836**
Percentage	**19.236***	**13.09686**	**3.819918**	**9.41337**	**14.32469**	**15.00682**	**14.59754**	**10.50477**	**100**

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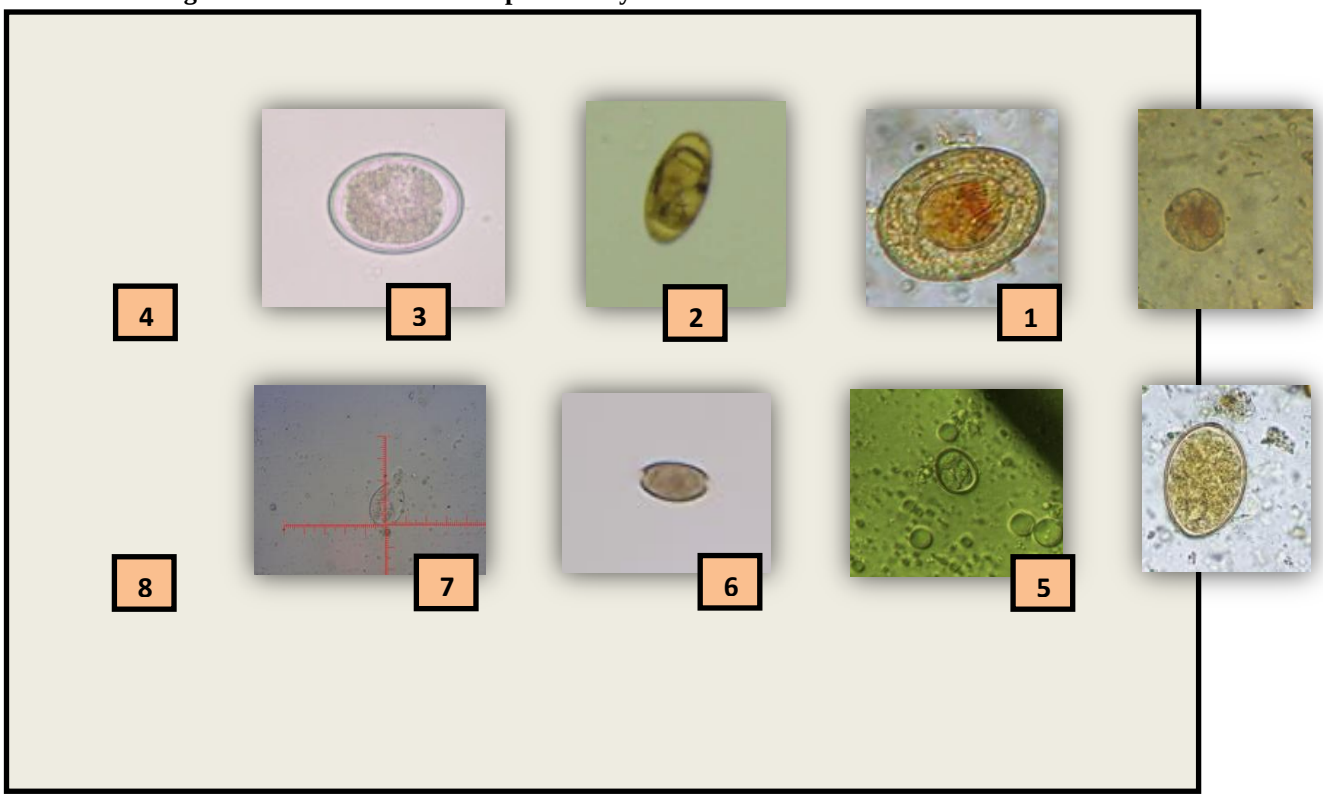
*** Indicate significant differences at 0.05 probability level**



Picture (3) shows the parasitic helminthes under study

1-*Ascaris lumbricoid* eggs. **2**-*Hymenolepis nana* eggs. **3**-*Fasciola hepatica* eggs. **4**-*Strongyloides stercoralis* eggs. **5**-*Toxocara spp.* eggs. **6**-*Trichuris trichura* eggs. **7**-*Ancylostoma duadenal* eggs. **8**-*Taenea spp. eggs*.

In addition, the diagnosis of *Cyclosporacatenensis*samples was confirmed by the use of the nPCR genetic method. Picture (5).

clear [16] that the molecular method to diagnose living organisms is one of the most accurate methods, as it took 6 types of vegetables (*perilla leaves*, winter-grown *cabbages*, *chives*, *sprouts*, *blueberries*, *and cherry* tomatoes) and it was examined and it was found that *Cyclospora* gave a percentage of pollution to vegetables (1.2%) , After examining a group of vegetables to detect parasites, [17]found that the best way to diagnose was the molecular method so as to ensure that fresh and edible vegetables by humans should be cleaned thoroughly to get rid of pathological parasites that are easily transmitted to the human body after eating it For contaminated vegetables.

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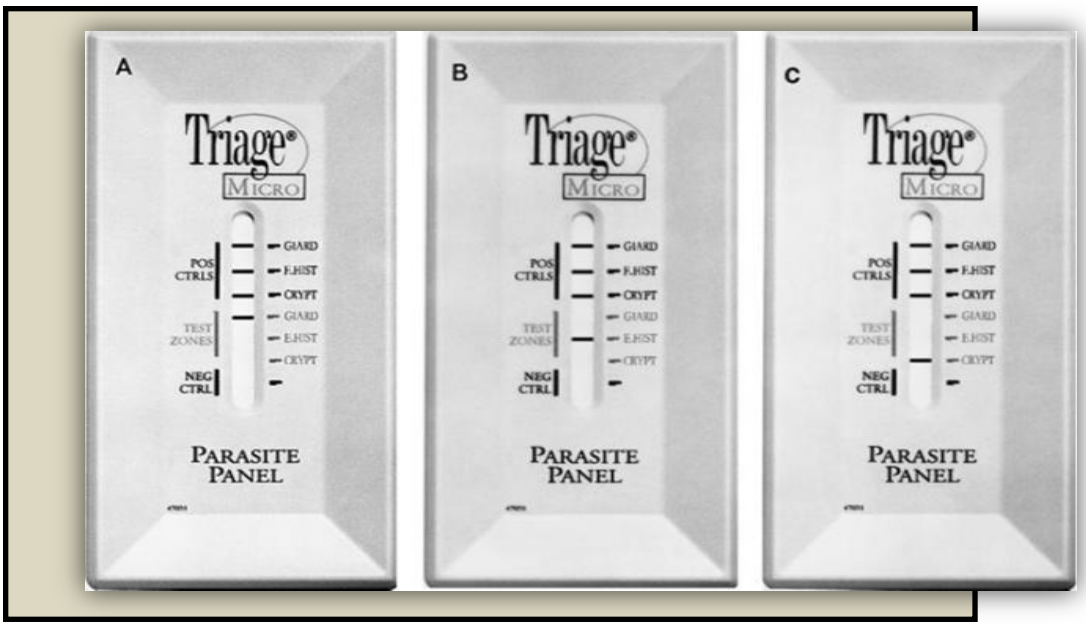
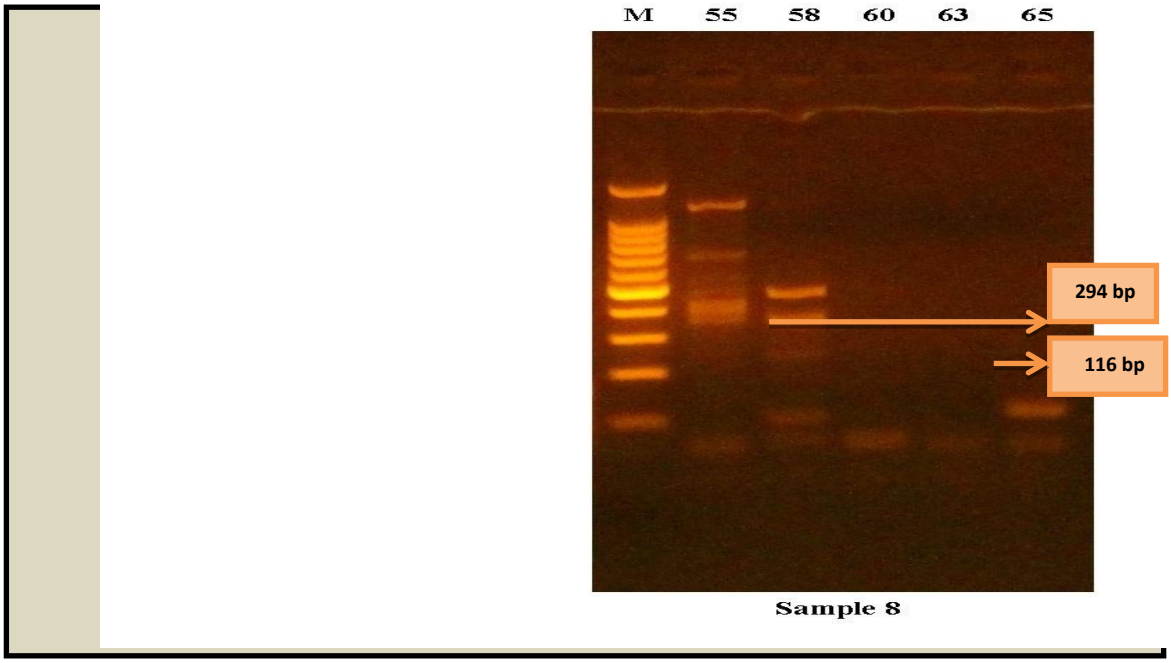


Picture (4) shows the examination of some parasitic protozoa using cassette



Picture (5) showing the large piece294bp and the small piece 116bp after migrating the sample on the Agarose 1%

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