International Journal of Medicine Research
ISSN: 2455-7404; Impact Factor: RJIF 5.42
www.medicinesjournal.com
Volume 1; Issue 5; November 2016; Page No. 07-13

Prevalence of intestinal parasites in fresh leafy vegetables in some farms at Dhamar city, Yemen

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Abstract
The present study has been done to assess the degree of contamination of fresh leafy vegetables with parasites in some farms in Dhamar city, Yemen. The results have shown that all 70 examined samples of the selected five types of vegetables were positive for 100% contamination with parasites. The examined vegetables *Allium porrum, Nasturtium officinale, Coriandrum sativum, Petroselium hortens* and *Lactuce sativa* were fresh leafy and they are commonly used daily as a green salad by consumers.
The intestinal parasites which have been found belong to protozoa and helminthes. The protozoan parasites which have been reported in this study are *Entamoeba histolytica* (26.9%), *Entamoeba coli* (18.2%), *Balantidium coli* (3.8%) and *Giaredia lamblia* (3.8%). The reported helminthes are *(Ascaris lumbricoides* (12.5%), *Hymenolepis nana* (1.9%), *Hymenolepis diminuta* (1.9%), *Enterobuse* (19.2%), *Taeniasa ginata* (6.7%), *Heterophyes heterophyes* (0.9%), *Fasciola hepatica* (2.8%) and *Hook worm* (0.9%). *Allium porrum* has shown contamination of (25%) which is the highest poly-parasitic contamination of twelve species of parasites followed by *Nasturtium officinale* (22.11%), *Coriandrum sativum* (18.2%) and *Petroselium hortens* (18.2%), whereas *Lactuce sativa* (16.3%) showed the least of poly-parasitic contamination of parasites.
The degree of contamination in 7 different chosen farms in Dhamar city has shown that the highest intensity contamination was found in (farm3, farm4 and farm5) with (17.3%) each, followed by farm1 (13.5%), farm 2 and farm 6 (12.5%) each where farm7 (9.6%) was the least contaminated farm.

Keywords: contamination, leafy vegetables, intestinal parasites, Dhamar city, Yemen

1. Introduction
Fresh vegetables are an important part of diet in every family. Vegetables are rich sources of small proportion of protein, fat and a relatively high proportion of vitamins (such as vitamins A, C and K), provitamins, including dietary minerals such as calcium, magnesium, potassium and iron), alpha and beta carotene, zea-xanthins, crypto-xanthins, phenolics, flavonoids as well as anthocyanins [1-2].
Recently, numerous literatures reported cases of food-borne diseases linked to fresh leafy vegetables. The consumption of Fresh leafy vegetables is a major way of the transmission of parasitic diseases to human beings [3].
One of the major factors influencing the prevalence of parasitic infections in the population is the habit and traditional popularity of eating fresh or uncooked foods [4]. Albeit, it is estimated that as much as 60% of the world's population is infected with intestinal parasites, which may be transmitted through direct and indirect contact with food, water, soil vertebrate and arthropod vectors and, rarely from mother to offspring [5-6].
Intestinal parasites are widely prevalent in developing countries; it is probably due to poor sanitation and inadequate personal hygiene [6]. There is a wide variety of food products that may be contaminated with one or more parasites and consequently transmitted to human beings. The prevalence of specific parasites in food supplies varies between countries and regions [4].
Fresh vegetables are one of the diseases producing agents, since many protozoan cysts, helminthes eggs and larvae can be transmitted by consuming the contaminated vegetables [7-9].
The aim of the present study is to assess the degree of contamination of fresh leafy vegetables with parasites in some farms in Dhamar city as a first study in Yemen and also to deal with the prevalence of parasitic infections in common vegetables that are used daily fresh such as (*Allium porrum, Coriandrum sativum, Petroselium hortens, Nasturtum officinale* and *Lactuce sativa*). These vegetables are commonly known as medicinal plants in Yemen [10].

2. Materials and Methods
Study Area
The present study was carried out in Dhamar city, Yemen; Dhamar is located between N 14.545545 latitude, E 44.408735 longitude and 2415 meters above sea level [11-12]. Dhamar map is shown in (Fig: 1). The temperature in Dhamar city is varied in different seasons (Summer, rainy and winter), the average temperatures range from 10 to 19 °C (50 to 66 °F) in summer, and from 8 to −1 °C (46 to 30 °F) in winter.

Study Design
From December 2015 to April 2016, seventy different vegetables samples *Allium porrum, Coriandrum sativum, Petroselium hortens Nasturtium officinale and Lactuce sativa* were collected from local farms, in Dhamar city with the help of the working people in the farms at the study area. These vegetables were chosen because they are fresh daily vegetables which are consumed by the people. 100 gms of each vegetable species were put in a nylon bag with their labeling details (date, place, time of collection and the common and scientific name of the vegetable). From each

species of the five experimental vegetables 14 samples of each species (2 samples from each farm) were collected. The total of 70 samples of all collected species was taken to the parasitic laboratory of Dhamar General Hospital to be examined.

Sample Examination

The samples were carefully washed with 100 ml of Normal saline separately in clean and sterile glass bottles. The bottles were left on the bench for six hours for proper sedimentation of the samples. The suspension was strained through a sterile sieve to remove unwanted materials and big particles. The filtered samples were put in test tubes separately and centrifuged at 3000 rpm for 3 minutes. The sedimentation was taken by pipette. One drop of the sediment is placed in a slide and observed under the light microscope (Olympus CX21i microscope). The cysts, eggs and larvae of parasites species were identified with the help of the parasitology expert in the Department of Parsitology in the same hospital. Each parasite, eggs, larvae or cysts present in the samples were counted.

The statistical analysis

Analyses were carried out using chi-square tests of the SPSS software version 8.1for windows (SPSS Inc., Chicago, IL, USA). The differences were considered significant at $p< 0.02$.

3. Results

The results of the present study on 70 examined samples of five species of vegetables have shown positive results with 100% of contamination with parasites.

The most contaminated species of vegetables is *Allium porrum* (25%) followed by *Nasturtium officinale* (22.1%), *Coriandrum sativum* (18.3%) and *Petroselium hortens* (18.3%), whereas the least contamination rate is in *Lactuce sativa* (16.3%). The frequency and percentage of contamination of vegetables are shown in (Table: 1 and Figure 2). Chi-Square Test has shown that there is no significant difference between vegetables in prevalence parasites (Table: 2 and Figure: 3).

The parasites observed in this study belong to protozoa and helminthes. Protozoan parasites *Entamoeba histolytica*, has shown the highest prevalence (26.9%) followed by *Entamoeba coli* (18.3%). Whereas the least ones *Balantidium coli* and *Giardia lamblia* (3.8%) each. While the helminthes were *(Enterobius vermicularis* (19.2%) the highest prevalence followed by *Ascaris lumbricoides* (12.5%), *Taenia saginata* (6.7%), *Fasciola hepatica* (2.9%) *Hymenolepis.nana* and *Hymenolepis diminuta* (1.9%) each, whereas the least were *Heterophyes heterophyes* (1.0%) and *Hook worm* (1.0%). The frequency and percentage of parasites prevalence are in Table: 3 and Figure: 4)

The highest species of protozoan parasite were observed in *Coriandrum sativum* (19.2%) followed by *Nasturtium officinale* (11.5%), *Allium porrum* (10.5%), *Coriandrum sativum* (8.6%) and *Lactuce sativa* (8.6%). More details on the comparison of number of parasites species and percentage observed in each species of the vegetable were shown in the table 4 and figure 5.

The most contaminated farms are farm 3, farm 4 and farm 5 which have the same percentage of contamination of (17.3%), followed by farm 1 which shown contamination percentage of (13.5%), while farm 2 and farm 6 have shown contamination of (12.5%) each, whereas the least contaminated farm is farm 7 which shown contamination of (9.6%).

Among the protozoan parasites observed in this study, *Entamoeba histolytica* was the most common parasite which was observed in farms 1, 2, 4 5, 6 and farm 7 with different levels of percentage of contamination. *Entamoeba coli* has shown the highest percentage is in farm 3 and 4, whereas the least percentage was observed in farm 1 and farm 7. *Balantidium coli* has shown the highest percentage is in the farm 1 and 5, but there was no contamination of *Balantidium coli* observed in other farms. *Giardia lamblia* has the highest percentage of contamination has been observed in farm 6 and 7 with different percentage, while in other farms no any contamination was observed. See table 4 and figure 5.

Among the helminthes parasites observed in this study, *Ascaris lumbricoide*s was the most common parasite which was observed in the farm 3, 5 and farm 6 followed by farm 7, while the least percentage was observed in farm 2 and 4, whereas no contamination was observed in the farm 1. *Hymenolepis nana* has shown the highest percentage in the farm 1 and 3; nevertheless there was no contamination observed in other remaining farms. *Hymenolepis diminuta* was observed only in the farm 4 and no contamination was observed in the other farms. In the same way *Enterobius vermicularis* has the highest percentage observed in the farm 2 and farm 3; while the least percentage of contamination was observed in the farm 5 and farm 6, but in the farm 7 there was no contamination observed. Furthermore, *Taenia saginata* has shown the highest percentage in farm 4 and farm 1 followed by farm 3 and 6, but in other farms there was no contamination with *Taenia saginata* was recorded. Farm 3 has shown the highest percentage of contamination with *Heterophyes heterophyes*, but in the other farms there was no contamination with *Heterophyes heterophyes* observed. *Fasciola hepatica* and *Hook worm* were observed only in the farm 5 and no contamination with *Fasciola hepatica* or *Hook worm* were observed in the other farms. More details with number of parasites and the percentage were shown in table 4 and figure 5. Chi-Square Test shows significant difference between farms in prevalence parasites P (0.02), df =66.

Fig 1: Map of Yemen shown Dhamar City (red color)

Table 1: Frequency and Percent of contamination of vegetables

Vegetables	Frequency	Percent
Allium porrum	26	25.0
Coriandrum sativum	19	18.3
Petroselium hortens	19	18.3
Nasturtium officinale	23	22.1
Lactuce sativa	17	16.3
Total	104	100.0

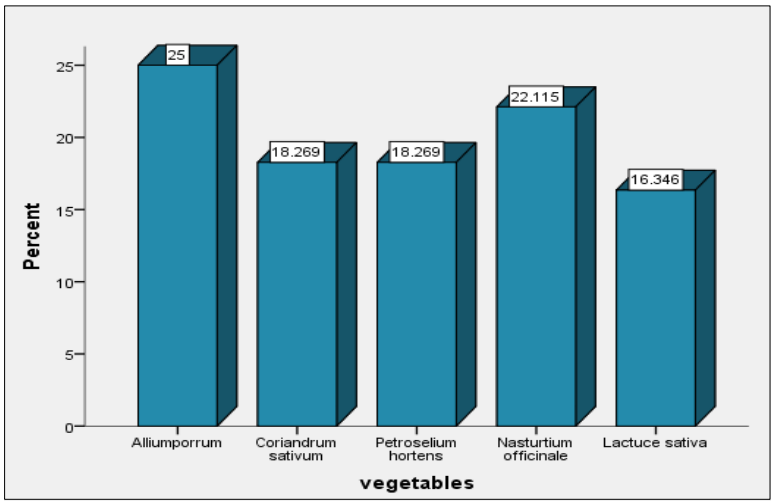


Fig 2: Percent of contamination of vegetables.

Table 2: Frequency and Percent of parasites prevalence.

Parasites	Frequency	Percent (%)
Entamoeba histolytica	28	26.9
Entamoeba coli	19	18.3
Balantidium coli	4	3.8
Giardia lamblia	4	3.8
Ascaris lumbricoides	13	12.5
Hymenolepis nana	2	1.9
Hymenolepis diminuta	2	1.9
Enterobius vermicularis	20	19.2
Taenia saginata	7	6.7
Heterophyes heterophyes	1	1.0
Fasciola hepatica	3	2.9
Hook worm	1	1.0
Total	104	100

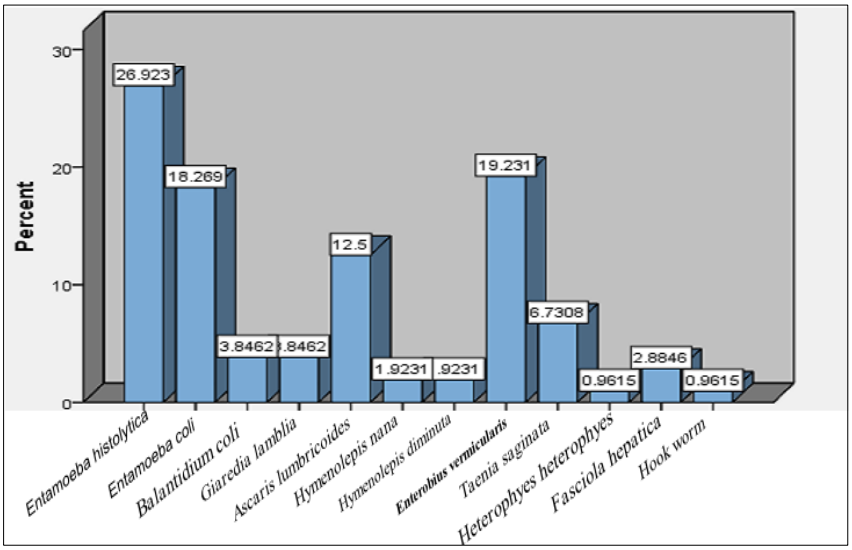


Fig 3: Percentage of parasites prevalence.

Table 3: Comparison of parasites species in examined vegetables

	Allium porrum		Coriandrum sativum		Petroselium hortens		Nasturtium officinale		Lactuce sativa		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Entamoeba histolytica	7	6.70	4	3.80	5	4.80	7	6.70	5	4.80	28	26.90
Entamoeba coli	4	3.80	6	5.70	3	2.80	3	2.80	3	2.80	19	18.20
Balantidium coli	0	0	2	1.90	0	0	1	0.90	1	0.90	4	3.80
Giardia lamblia	0	0	2	1.90	1	0.90	1	0.96	0	0	4	3.80
Ascaris lumbricoides	4	3.80	1	0.90	3	2.80	4	3.80	1	0.90	13	12.50
Hymenolepis nana	1	0.90	0	0	0	0	1	0.90	0	0	2	1.90
Hymenolepis diminuta	0	0	1	0.90	1	0.90	0	0	0	0	2	1.90
Enterobius vermicularis	5	4.80	2	1.90	3	2.80	4	3.80	6	5.76	20	19.20
Taenia saginata	3	2.80	1	0.90	2	1.90	1	0.90	0	0	7	6.70
Heterophyes heterophyes	0	0	0	0	0	0	1	0.90	0	0	1	0.90
Fasciola hepatica	1	0.90	0	0	1	0.90	0	0	1	0.90	3	2.80
Hook worm	1	0.90	0	0	0	0	0	0	0	0	1	0.90
Total Percent	26	14.40	19	4.80	19	9.60	23	10.50	17	7.60	104	100

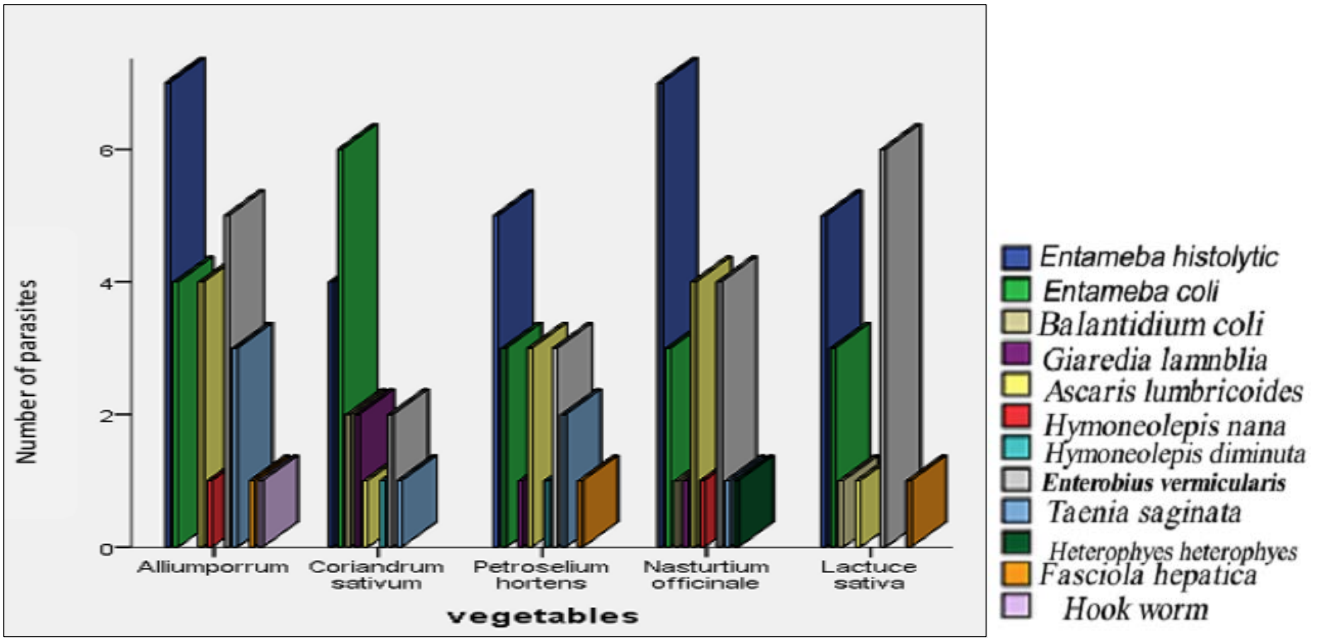
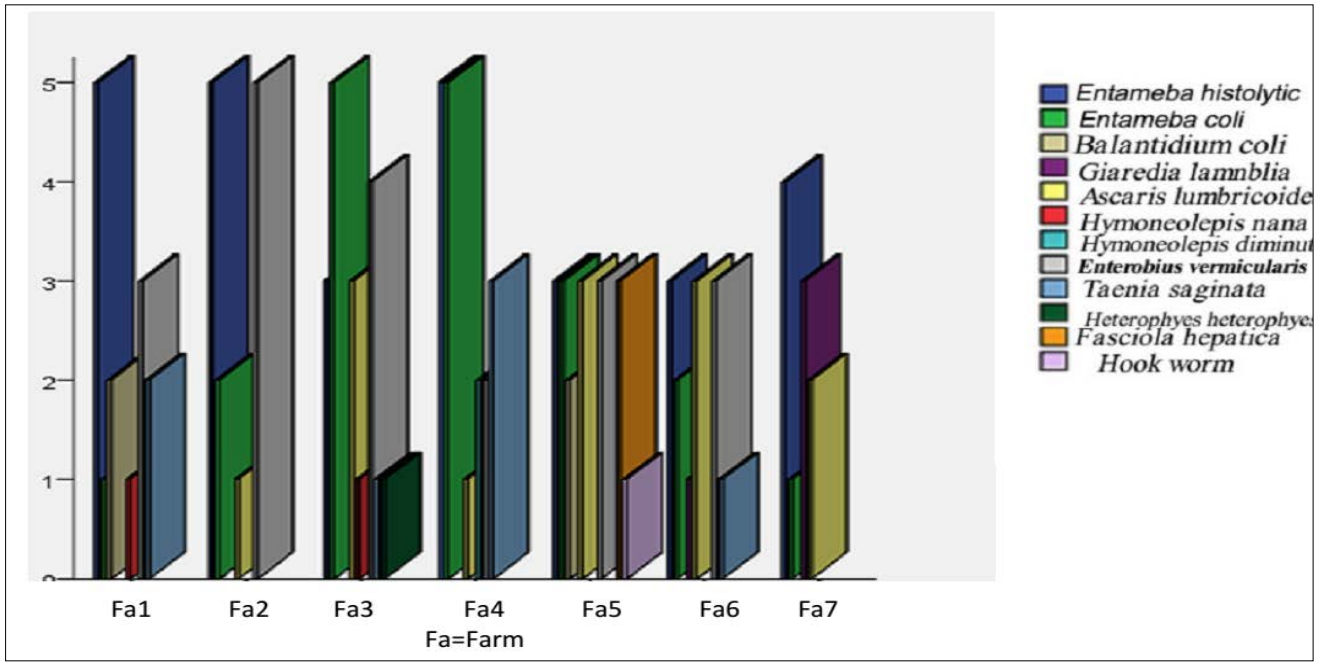


Fig 4: The difference of parasites number according to species of vegetables

Table 4: Comparison between parasitic contaminations in 7 farms in Dhamar city

	*Fa1		Fa2		Fa3		Fa4		Fa5		Fa6		Fa7		Total	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
Entamoeba histolytica	5	4.80	5	4.80	3	2.80	5	4.80	3	2.80	3	2.80	4	3.80	28	26.90
Entamoeba coli	1	0.90	2	1.90	5	4.80	5	4.80	3	2.80	2	1.90	1	0.90	19	18.26
Balantidium coli	2	1.90	0	0	0	0	0	0	2	1.90	0	0	0	0	4	3.80
Giardia lamblia	0	0	0	0	0	0	0	0	0	0	1	0.96	3	2.80	4	3.80
Ascaris lumbricoides	0	0	1	0.90	3	2.80	1	0.90	3	2.80	3	2.80	2	1.90	13	12.50
Hymenolepis nana	1	0.90	0	0	1	0.90	0	0	0	0	0	0	0	0	2	1.90
Hymenolpis diminuta	0	0	0	0	0	0	2	1.90	0	0	0	0	0	0	2	1.90
Enterobius vermicularis	3	2.80	5	4.80	4	3.80	2	1.90	3	2.80	3	2.80	0	0	20	19.20
Taenia saginata	2	1.90	0	0	1	0.90	3	2.80	0	0	1	0.96	0	0	7	6.70
Heterophyes heterophyes	0	0	0	0	1	0.90	0	0	0	0	0	0	0	0	1	0.90
Fasciola hepatica	0	0	0	0	0	0	0	0	3	2.80	0	0	0	0	3	2.80
Hook worm	0	0	0	0	0	0	0	0	1	0.96	0	0	0	0	1	0.90
Total	14	13.50	13	12.50	18	17.30	18	17.30	18	17.30	13	12.50	10	9.60	104	100

*Fa= Farm, Fa1=Region 1, Fa2= Region 2, Fa3= Region 3, Fa4= Region 4, Fa5= Region 5, Fa6= Region 6, Fa7= Region 7



*Fa= Farm, Fa1= Region 1, Fa2= Region 2, Fa3= Region 3, Fa4= Region 4, Fa5= Region 5, Fa6= Region 6, Fa7= Region 7

Fig 5: comparison between parasitic contaminations in 7 farms in Dhamar city.

4. Discussion

In developing countries, such as Yemen, intestinal parasites infestations are very common. Consumption of fresh vegetables is a major way in the transmission of parasitic contaminations. Our study revealed the presence of protozoa and helminthes in the analyzed samples with an average (100%) that means all vegetables samples examined were positive. This percentage was the highest report among the other previous studies from different countries, in Tabriz-Iran contamination percentage reported (76%) [3], also in Khorramabad- Iran reported 79% (52.8% in spring and 26.2% in winter) [13], in Nigeria two different studies reported (56.2% and 40.7%) respectively [14-15], in Lahore- Pakistan (31.2%) [16], in Alexandria- Egypt (31.7%) [17] and in Kufa-Iraq (20.5%) [18]. Lower rates of contamination in the Middle East were reported in Riyadh, Saudi Arabia (16.2%) [19], while in Turkey (6.3%) [20].

In the current study we have isolated twelve species of parasites, four species belong to protozoa (*E. histolytica, E. coli, B. coli, G. lamnblia*) and eight species belong to helminthes (*A. lumbricoides, H. nana, H. diminuta, E. vermicularis, T. saginata, Hook worm, F. hepatica* and *H. heterophyes*). Most of these isolated species were recorded in several previous studies in different places [3, 21-22].

Biologically, the highest health risk is for helminthic infections compared with other pathogens because helminthes persist for longer periods in the environment and the infective dose is small [23]. Helminthes and Protozoa are among the largest group of parasites that inhabit the human intestine [24].

Entamoeba histolytica was the most prevalent protozoa (26.9%). It really has shown highest percentage recorded parasite because it was observed in all samples of the examined vegetables; such result of high percentage (25.30%) recorded in Al-Nassiriyah city-Iraq [25], while lower percentage were record in Al-Qassim Region, Saudi Arabia (4.4%) [22], and (5.8%) reported in Ghana [21]. The reasons behind that maybe related to human faeces outside the home or near to the farms which are transmitted to vegetables especially faeces of children in open areas. Some of previous studies reported that *Entamoeba histolytica* is the most prevalence of parasites in pre-school and schools children [26-27].

Entamoeba coli was the second prevalence of protozoan parasite which recorded (18.2%); while some studies reported (23.7%) in Al kufa city- Iraq [18], in Zahedan city- Iran reported (29.3%) [28], while in Khorramabad- Iran researchers reported 12.3% (8.4% in spring and 2.9% in winter) [13].

Enterobius vermicularis was the most prevalent helminthes (19.2%) recorded in this study, while in Khorramabad- Iran study reported 5.1% (4% in spring and 1.1% in winter) [13] and in Zahedan city- Iran (8.1%) was recorded [28], which is lower than our result. *Enterobius vermicularis*o ova can also enter the digestive tract by ingestion of vegetables and food products, as the main source of direct transmission of intestinal parasites [28].

In Alexandria- Egypt, one study reported (20.3%) contamination of some vegetables with *Ascaris lumbricoides* eggs [17], this result is higher than the result reported in the current study which detected a percentage of contamination of (12.5%). In Iran some studies reported the rates of contamination with Ascaris eggs in vegetables were (2.5%, 2%, 2.3% and 6.1%) in Jiruft, Ardabil, Qazvin and in Zahedan respectively [29-31, 28]. Moreover, one study was conducted in Khorramabad and reported 5.8% (4.7% in spring and 1.1% in winter). In the same study in Khorramabad, the higher contamination rate with Ascaris eggs was detected in green onion samples followed by leek among the examined vegetables in spring season [13].While in Plateau State- Nigeria one study reported (2.4%) contamination [14]. But the highest prevalence percentage contamination of *Ascaris lumbricoides* in consumed vegetables was (26.3%) reported from Riyadh- Saudi Arabia [19]. Ascariasis is prevalent worldwide especially in tropical and subtropical countries. Infections with these parasites are

more common in the areas where the sanitation, personal hygienic is poor and the places in which the raw human faeces are used as fertilizer [32].

The most contaminated species among the examined vegetables was *Allium porrum* (25%), similar result of highest contamination of (41%) reported in Iraq [18]. *Nasturtium officinale* contamination detected was (22.1%) in current study which is lower than percentage reported in Alexandria- Egypt (46.7%) [17]. *Petroselium hortens* has shown (18.3%) contamination in the current study, while some studies reported lower percentage of (10.4%) [18]. In Pakistan, (8.9%) contamination in *Coriandrum sativum* was reported [16], which is lower than our report in the current study in which we detected (18.3%) contamination in the examined samples, while in Mannuthy, Kerala state-India, no contamination of any intestinal parasites in *Coriandrum sativum* was reported [33].

Lactuce sativa, in our study has shown (16.6%) which was the lowest contaminated species among the chosen vegetables, but another study in Al-Qassim Region- Saudi Arabia has shown highest recorded (20.6%) of contamination [22].

Generally, there are many reasons of parasitic infection such as the type of water used for irrigation vegetables like wasted or contaminated water used for irrigation, organic fertilizers (sewage sludge, animal manure and compost) and sanitary conditions in the areas where the vegetables are cultivated may also have a potential risk of contamination of vegetables [34-36, 13]. In addition to that, the eaten part of leafy vegetable attaches with contaminated soil, so the parasites can be transported to the vegetables simply during the irrigation. Climate and season are very important source of growing and reproduction of parasites. Ezatpour [13] reported the high contamination occurred in the spring season more than in winter. Farmers' and school children's bad habits like excreting near to the farms are considered a real factor to transmission of the parasites. Many different animals belong to the farmers (dogs, cats, cows and sheep inside the farms) which have been seen in many farms in the study area which are intermediate factors for transmission of some species of parasites. We conformed that farms (3, 4 and 5) were irrigated by wasted water from surrounding polluted areas so that these farms have shown the highest contamination level among the all seven farms.

5. Conclusion

The findings in our study may have important implications for the global food safety and highlight the importance of raw vegetables in ominous public health by transmission of intestinal parasites to humans in Dhamar City- Yemen. The local healthcare and environmental authorities should improve the sanitized conditions in the areas where the vegetables are cultivated. Appropriate treatment of wastewater used for irrigation of vegetables must be implemented. More researches are required to screen of parasitic contamination in green vegetables in other cities in Yemen. Similarly, researches must be performing to evaluate the level of contamination water used for irrigation and soil in which green vegetables used to be cultivated.

At homes and restaurants, vegetables should be soaked and washed well before consumption. Moreover, personal hygienic for vegetable handlers and transporters should be taken into consideration.

6. Acknowledgement

The authors are thankful to the laboratory staff members in the Department of Parasitology of Dhamar General Hospital, Dhamar city, Yemen for providing the laboratory facility.

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