



RESEARCH ARTICLE

PARASITIC CONTAMINATION OF VEGETABLES IN SELECTED LOCAL MARKETS IN ADEN GOVERNORATE, YEMEN

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Abstract

Fresh and raw vegetables may play a significant role in transmission of intestinal parasitic infections to humans. Therefore, this study was conducted to estimate the level of parasitic contamination in vegetables which are consumed raw in Aden governorate, Yemen. This is a descriptive analytic cross-sectional study in which six local markets were randomly selected. A total of 216 fresh vegetable samples including parsley (*Petroselinum sativum*), watercress (*Nasturtium officinale*), lettuce (*Lactuca sativa*), leek (*Allium porrum*), green onion (*Allium cepa*), and tomatoes (*Solanum Lycopersicum*) were purchased from the selected markets and analysed using direct and iodine wet mount smear preparation. Modified Ziehl–Neelsen staining and Zinc sulphate flotation method were also used. Smears were examined by under the light microscope. Out of 216 vegetable samples, 166 (76.9%) were contaminated with one or more parasites. Parsley was the most contaminated (97.2%), followed by watercress (94.4%), lettuce (88.9%), leek (80.6%), green onion (77.8%), whereas the least contamination rate was in tomato (22.2%). Statistically significant difference in parasitic contamination rate was found between different types of vegetables ($P < 0.05$). The most common parasite was *Ascaris lumbricoides* 75 (24.4%), followed by cyst of *Entamoeba histolytica/dispar* 64 (20.9%), and *Entamoeba coli* 52 (16.8%). Significant difference was reported in prevalence rate between different parasitic species ($P < 0.05$). Vegetable samples collected from Dar Saad market, show the highest contamination rate (94.4%) while vegetables collected from Al Mansura market showed the lowest contamination rate (50.0%). However, the parasitic contamination rates were not significantly different for samples collected from different markets. This study highlighted the importance of raw vegetables as the potential source of transmission for intestinal parasites to humans. Prevention methods such as proper washing or cooking of vegetables before consumption should be conveyed to consumers. In addition, comprehensive health education and hygienic practices, including washing hands after handling vegetables, should be provided to sellers and consumers.

Keywords: Vegetables, Contamination, Intestinal Parasites, Markets.

Introduction

Intestinal parasites are one of the biggest public health problems worldwide, especially in tropical and subtropical countries. Globally, it is estimated that 3.5 billion people are affected, 450 million people becoming infected with foodborne parasites, and about 200 000 people die annually [1, 2]. Yemen is an underdeveloped country plagued by poverty, diseases, and social conflicts. Also, most of the population lives in rural areas and is vulnerable to intestinal parasite infections [3]. They are known to cause diarrhea, iron deficiency

anemia, infant growth retardation, physical weakness, low educational performance, and other physical and mental health problems [4]. Intestinal parasites are primarily transmitted by fecal-oral route, mostly via ingestion of contaminated food and water or during direct hand to mouth contact [5]. Vegetables and fruits can become contaminated with parasites throughout the process from planting to consumption [6]. In the pre-harvest stage, contamination of vegetables may occur due to the use of human and animal manure as natural fertilizers and the irrigation of vegetables with untreated

wastewater. In developing countries, most local farmers use untreated human and animal waste as fertilizer and polluted water for irrigation, contributing to increased transmission of intestinal parasites [7]. Hygienic standards for storage, transportation, and marketing conditions, catering and processing for consumption play an important role in the post-harvest stage [8]. In addition, food items such as vegetables and fruits which are usually consumed raw, or lightly cooked in order to retain the natural taste and to preserve heat labile nutrients are potential sources of parasitic infection, and this practice facilitates the transmission of food-borne infections [2, 8, 9]. On the other hand, Yemen is a country with many intestinal parasitic infections due to lack of clean water and sanitation and as a result of open defecation is expected to contaminate farmland with infectious intestinal parasites [3, 10, 11]. All of these factors lead to parasitic contamination of vegetables and fruits, making these foods important vectors of transmission to humans [12]. Moreover, studies conducted on various items of vegetable and fruit samples have shown that *Ascaris lumbricoides*, *Entamoeba histolytica/dispar*, *Giardia intestinalis*, *Enterobius vermicularis*, *Cryptosporidium* spp, *Hymenolepis nana*, *Taenia* spp, *Trichuris trichiura*, *Cyclospora* spp, *Fasciola hepatica*, and hookworms infect humans who consume contaminated vegetables and fruits without cooking or washing them properly [2,10, 13-17]. However, the rate of contamination and the detected species of parasites vary by weather conditions, socio-cultural status, season of sample collection, and type and number of vegetable items examined and other factors [17]. Also, epidemiological studies indicated that the number of reported cases of food-borne illnesses due to consumption of raw vegetables and fruits has been increasing and continues to be a common and serious threat to public health in endemic areas for intestinal parasitic infections [12,14]. There are limited studies on the possible contamination of vegetables in Yemen, and the recent study conducted in Dhamar city, Yemen,

showed that all examined samples of the selected vegetables were positive for 100% contamination with parasites [10]. This demand monitoring of the contamination status at local settings in order to intervene in transmission of intestinal parasitic infections. Despite this, to our knowledge, there is no published document on vegetable contamination with medically important protozoans and helminths in Aden governorate. Hence, the aim of the present study was to determine the parasitic contamination level of vegetables in selected local markets of Aden governorate, Yemen.

Methods

Study Design, Area and Period

A descriptive analytic cross-sectional study was conducted in six selected local markets (Al Mansura, Craiter, Al Buraiqeh, Al Shaikh Outman, Attawahi, and Dar Saad markets) in Aden governorate, Yemen, from July to December 2022. Aden governorate map is shown in [Figure1] [18]. Aden governorate is a large port and a key resort city located at 12° 48' North latitude and 45° 1' East longitude [19]. It is has an area of 518.5 km² holding a population of around 966,424. In Aden governorate, people practice, different activities, industrial, fishing, commercial and services. Agriculture and animal husbandry are not considered as the main activities. Geographically, it is consists of 8 directorates; Craiter, Al-Mualla, Attawahi, Khor Maksar, Al Buraiqeh, Dar Saad, Al Mansura, and Sheikh Othman [18]. Over the course of the year, the temperature typically varies from 74°F to 95°F (23.3 °C to 35 °C) and is rarely below 73°F (22.8°C) or above 98°F (36.7°C). Aden governorate experience two distinct, hot and wet season which occur from July to September with an average daily high temperature above 92°F(33.3°C) followed by a lesser cold and dry season in October to December with an average daily high temperature below 84°F(28.9°C) [20].

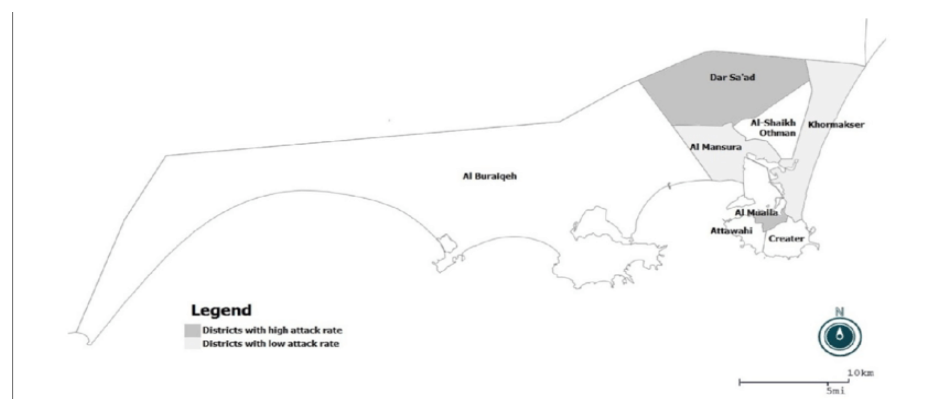


Fig. 1: Map of Aden governorate [18].

Sample Collection, Processing and Parasite Detection

A total of 216 fresh and raw vegetable samples of 6 different types that are frequently consumed were purchased from six randomly selected markets. An equal number of samples (36 each, 216 total) were collected from each market and a single vegetable sample was purchased from each vendor. The fresh vegetable samples included in this study were parsley (*Petroselinum sativum*), watercress (*Nasturtium officinale*), lettuce (*Lactuca sativa*), leek (*Allium porrum*/*Allium ampeloprasum*), green onion (*Allium cepa*), and tomatoes (*Solanum Lycopersicum*). Fresh and raw vegetables were collected into sterile, labeled (sample type, date of collection and name of market) polythene bags and transported immediately to the Quality Control Laboratory at Microbiology Department - Supreme Board of Drugs and Medical Appliance, Aden, for parasitic examination.

To detect the presence of parasites in the studied samples, we followed a standard protocol explained elsewhere [21, 22, 23]. About 250 grams was weighted from each sample, and then each vegetable sample was washed in a separate sink containing 500mL physiological saline (0.9 % Sodium chloride solution) for detaching the parasitic stages (ova, larvae, cysts, and oocysts) of helminths and protozoan parasites commonly assumed to be associated with vegetable contamination. After overnight sedimentation of the washing solution, 15mL of the sediment was then transferred to a centrifuge tube using sieve, to remove undesirable matters. For concentrating the parasitic stages, the tube was centrifuged at 3000 rpm for five minutes. After centrifugation, the supernatant was decanted carefully without shaking. Then the sediment was agitated gently by hand for redistributing the parasitic stages. From the sediment unstained wet mount smear and Lugol's iodine stained smear were prepared. For unstained smears, a drop of sediment was placed on a fresh clean slide and a coverslip was placed carefully to avoid air bubbles and flooding. Stained smears were prepared as per the unstained smears but Lugol's iodine solution was added. Finally, these smears were examined under a light microscope using $\times 10$ and $\times 40$ objectives.

Modified Zeihl-Neelsen staining technique was also used for identification of oocysts of *Cryptosporidium* and *Cyclospora spp.* The Ziehl-Neelsen stain was modified by replacing sulphuric acid with acid-alcohol decolourising agent (3% hydrochloric acid in 95% ethanol) and using Malachite green stain as a counterstain to provide differential staining for coccidian parasites such as *Cryptosporidium* oocysts. Oil

immersion lens ($\times 100$) was used to exam stained smears with Ziehl-Neelsen stain. The zinc sulfate flotation method (specific gravity 1.18) was also used to detect helminth eggs and protozoan cysts. Parasite stages (eggs, cysts, larvae, and oocyst) were identified based on morphological details as described by WHO (1994) and Soulsby [24, 25]. Personal safety precautions were taken during every procedure and the microscopical parasite identification was carried out by skilled medical laboratory personnel. Four slides were prepared and examined per sample by unstained wet mount, iodine smear, Zinc sulphate flotation method, and Modified Ziehl-Neelsen staining technique.

Ethical Considerations

1. An ethical approval letter was obtained from Research and Ethical Review Committee, Faculty of Science, Department of Zoology and Biology, University of Aden.
2. Verbal informed consent was obtained from each study participants (vendors).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 20.0 was used for testing the statistical relationship between and within variables. Descriptive statistics like frequencies and proportions of prevalence and distribution of parasites and contamination were used to summarize the data. Chi square test and analysis of variance ANOVA were used to determine significant difference in the prevalence and distribution of parasites in different vegetables and markets. Significant level was set at $P < 0.05$.

Results

Overall parasitic contamination of vegetables

The results of the current study showed that 166 out of 216 of vegetable samples were identified to be contaminated with at least one type of parasite, which gave rise to the overall contamination rate of 76.9%, as seen in table (1). Also, the table shows the frequency of distribution of each vegetable contamination. The most contaminated species of vegetables was parsley (97.2%), followed by watercress (94.4%), lettuce (88.9%), leek (80.6%), green onion (77.8%), whereas the least contamination rate was in tomato (22.2%). The parasitic contamination rate of the different vegetables was significantly different ($P = 0.027$). Further analysis with Scheffe test showed that the mean difference was significant at the .05 level (Table1).

Table 1: Frequency distribution of parasitic contaminations of vegetables

Type of Vegetable	No. Examined	No. Positive (%)	No. of Parasitic SPP. Detected (%)			
			Single	Double	Triple	Quadruple
Parsley	36	35 (97.2)	11	8	10	6
Watercress	36	34 (94.4)	14	11	5	4
Lettuce	36	32 (88.9)	17	8	4	3
Leek	36	29 (80.6)	14	9	6	0
Green onion	36	28 (77.8)	19	9	0	0
Tomatoes	36	8 (22.2)	2	4	2	0
Total	216	166 (76.9)	77 (46.4)	49 (29.5)	27 (16.3)	13 (7.8)

* A statistically significant difference in contamination rate between different types of vegetables ($P = 0.027$).** Also, the mean difference is statistically significant at the .05 level. (Scheffe test).

Multi-parasitic contamination of vegetables

In this study, contamination with more than one parasitic species was observed in the examined vegetable samples. Out of 166 contaminated vegetable samples, 77 (46.4%) of samples were contaminated with one type of parasite, 49 (29.5%) of samples were contaminated with 2 parasites, 27 (16.3%) of samples were contaminated with 3 parasites, and 13 (7.8%) of vegetables were contaminated with 4 parasites. The parasitic contamination rate of the different vegetables was significantly different ($P = 0.027$) (Table 1).

Frequency distribution of intestinal parasites in vegetable samples

In this study, both protozoa and helminth parasites were detected as contaminants of vegetables. A total of 14 parasites comprising of five protozoans (35.7%) and nine helminths (64.3%). Table 2 shows that *Ascaris lumbricoides* (24.4%) was the most frequently detected helminthic parasite followed by the *Entamoeba histolytica /dispar* (20.9%) that was the most frequently identified protozoa, followed by *Entamoeba coli* (16.8%), *Giardia intestinalis*(9.7%), *Hymenolepis nana* (6.8%), *Cryptosporidium spp.*(5.5%), *Enterobius vermicularis* (3.7%), *Strongyloides stercoralis*(2.6%), *Taenia spp.*(1.9%), *Trichuris trichiura*(1.6%), *Isospora spp.*(1.3%), *Fasciola hepatica* (0.6%), *Hook worm spp.*(0.6%), and unidentified parasitic larvae (3.6%) were also observed. Significant difference was reported in prevalence rate between different parasitic species ($P < 0.05$); In addition, Scheffe test showed significant difference in prevalence rate of *Ascaris lumbricoides*, *Entamoeba histolytica /dispar*, and *Entamoeba coli* compared with other parasitic species, as the mean difference was significant at the 0.05 level (Table 2).

Table 2: Frequency distribution of intestinal parasites in vegetables sold at six markets in Aden governorate from July to December 2022

Detected Parasite Species	Frequency	Prevalence
<i>Ascaris lumbricoides</i>	75	24.4
<i>Entamoeba histolytica /dispar</i>	64	20.9
<i>Entamoeba coli</i>	52	16.8
<i>Giardia intestinalis</i>	30	9.7
<i>Hymenolepis nana</i>	21	6.8
<i>Cryptosporidium spp.</i>	17	5.5
<i>Enterobius vermicularis</i>	11	3.7
<i>Strongyloides stercoralis</i>	8	2.6
<i>Taenia spp.</i>	6	1.9
<i>Trichuris trichiura</i>	5	1.6
<i>Isospora spp.</i>	4	1.3
<i>Fasciola hepatica</i>	2	0.6
<i>Hook worm spp.</i>	2	0.6
Parasitic larvae	11	3.6
Total sample (n = 216)		100

Abbreviation: spp, species. A statistically significant difference in prevalence rate between different parasitic species ($P < 0.05$); *** The mean difference was statistically significant at the .05 level (Scheffe test).

Frequency of parasitic contaminants among the six examined vegetables

Among examined vegetables, parsley was the most contaminated (81 samples; 26.3%) followed by watercress (67 samples; 21.8%), lettuce (57 samples; 18.5%), and tomato was found to be the least contaminated (16 samples; 5.2%). *Ascaris lumbricoides* was found to be the highest contaminant in parsley (17; 50.0%), followed by *Entamoeba histolytica/dispar* (17; 47.2%), and *Entamoeba coli* (14; 83.9%). Also, both *Ascaris lumbricoides* (16; 44.4%) and *Entamoeba histolytica/ dispar* (15; 41.7%) were the major contaminants in watercress. Table (3).

Table 3: Distribution of intestinal parasites in relation to the type of vegetable samples collected from six local markets in Aden governorate from July to December 2022

Vegetables Parasites	Parsley	Watercress	Lettuce	Leek	Green Onion	Tomatoes	Total	X ²	P value
<i>Ascaris lumbricoides</i>	17(50.0)	16(44.4)	14(83.9)	12(33.3)	11(30.6)	5(13.9)	75(34,7)	3696	P < 0.05
<i>Entamoeba histolytica/dispar</i>	17(47.2)	15(41.7)	10(27.8)	9(25.0)	8(22.2)	5(13.9)	64(29.6)	3696	
<i>Entamoeba coli</i>	14(83.9)	12(33.3)	10(27.8)	8(22.2)	6(16.7)	2(5.6)	52(24.1)	3696	
<i>Giardia intestinalis</i>	8(22.2)	6(16.7)	6(16.7)	4(11,1)	4(11,1)	2(5.6)	30(13.9)	2464	
<i>Hymenolepis nana</i>	5(13.9)	4(11,1)	3(8.3)	4(11,1)	4(11,1)	1(2.8)	21(9.7)	2464	
<i>Cryptosporidium spp.</i>	4(11,1)	3(8.3)	4(11,1)	3(8.3)	2(5.6)	1(2.8)	17(7.9)	2464	
<i>Enterobius vermicularis</i>	3(8.3)	2(5.6)	2(5.6)	3(8.3)	1(2.8)	0	11(5.1)	2464	
<i>Strongyloides stercoralis</i>	2(5.6)	1(2.8)	2(5.6)	2(5.6)	1(2.8)	0	8(3.7)	1848	
<i>Taenia spp.</i>	2(5.6)	1(2.8)	2(5.6)	1(2.8)	0	0	6(2.8)	1848	
<i>Trichuris trichiura</i>	2(5.6)	1(2.8)	1(2.8)	1(2.8)	0	0	5(2.3)	1848	
<i>Isospora spp.</i>	2(5.6)	2(5.6)	0	0	0	0	4(1.9)	1232	
<i>Fasciola hepatica</i>	1(2.8)	1(2.8)	0	0	0	0	2(0.8)	1232	
<i>Hook worm</i>	1(2.8)	1(2.8)	0	0	0	0	2(0.8)	1232	
Unidentified parasitic larvae	3(8.3)	2(5.6)	3(8.3)	3(8.3)	0	0	11(5.1)	848	
Total	81(26.3)	67(21.8)	57(18.5)	50(16.2)	37(12.0)	16(5.2)	308	3696	
X²	2156	2156	2156	2156	1848	924	3388		
P value	P < 0.05								

*Abbreviation: spp, species **The parasitic contamination rate of the different vegetables was significantly different (P < 0.05); ***The mean difference was statistically significant at the .05 level (ANOVA; Scheffe test).

The parasitic contamination among different markets

In the current study, the vegetable samples were collected from six local markets in Aden governorate. The results of the study showed that vegetable samples collected from “Dar Saad” (94.4%) had the highest contamination rate followed by samples collected from “Attawahi” (91.7%), “Al Shaikh Outman” (83.3%), “Al Buraiqeh” (80.6%), and “Craiter” (61.1%), while the lowest contamination rate was from “Al Mansura” (50.0%) markets. However, ANOVA test showed insignificant difference between markets among vegetable samples collected from different markets (Table 4).

Table 4: Number and percentage of contaminated vegetable samples according to the selected local markets in Aden governorate

Local Markets	Total Number	Positive Samples	
		N	%
Al- Mansura	36	18	50.0
Craiter	36	22	61.1
Al- Buraiqeh	36	29	80.6
Al -Shaikh Outman	36	30	83.3
Attawahi	36	33	91.7
Dar Saad	36	34	94.4
Total	216	166	76.9

*No significant difference in contamination rate among vegetable samples collected from the different markets.

Discussion

The detection of medically important intestinal parasites from vegetables suggests that vegetables are potential sources of foodborne illness in humans and also is an indicator of the fecal contamination from human and or animal origin. Their presence in the vegetables is associated not only with climatic conditions favorable to the existence and spread of parasites, but also with sanitary conditions and hygienic practices that facilitate their transmission [26].

In the present study, the overall parasitic contamination rate was found to be 76.9%, which is in agreement with the findings reported in Khorramabad, Iran, which reported an overall prevalence of 79% [27], Kisii Municipality, Kenya (75.9%) [28], and Tripoli, Libya (68%) [29]. However, this rate was higher than that reported in Thailand (35.1%) [30], Shahrekord, Iran (32.6%) [31], and Lahore- Pakistan (31.2%) [32]. On other hand, it is lower when compared with the findings from Akure, Southwestern Nigeria (88.8%) [33]. However, differences in contamination rates could be explained by variations in geographical locations, climatic and environmental conditions, the kind of sample and sample size examined, the sampling techniques, methods used for detection of the intestinal parasites, and socioeconomic status [34]. In addition, the differences between this study and others might be also attributed to the types of soil, types of water used for

agriculture, and poor hygienic practices during the transportation and marketing or farming of vegetables [35]. The obtained results in the present study indicate that the contamination of raw vegetables (76.9%) with pathogenic parasites in Aden governorate, Yemen, might represent a transmission vector for intestinal parasites to consumers. Thus, prevention methods such as proper washing or cooking of vegetables before consumption should be conveyed to consumers as well as vendors.

In the present study contamination with more than one parasite species was observed in the contaminated vegetable samples; about half (77;46.4%) were contaminated with one parasite, while 49 (29.5%), 27 (16.3%), and 13 (7.8%) were contaminated with 2, 3, and 4 parasites, respectively. In addition, the parasitic contamination rate of the different vegetables was significantly different ($P = 0.027$) Table (1).

This finding is in line with what was reported by other researchers where contamination by one type of parasite was higher than the double, triple and, quadruple ones. In a study performed by Tsegahun *et al.*, in Debre Berhan Town, Ethiopia, of the 180 samples examined, (75; 41.7%) were contaminated with at least one type of parasite. In addition, (37; 20.6%) and (23; 12.8%) vegetables were contaminated with 1 and 2 parasites, respectively [17]. In another study, out of 150 contaminated samples, 119 (79.3%) and 25 (16.7%) were contaminated with one and two parasite species, respectively [21]. Also, Tefera *et al.* [34], reported that single contamination is more common than the double and triple one. However, the presence of multiple parasitic species contamination of vegetables might indicate the possibility of high level contamination of the vegetables, which perhaps results in multiple parasitic infections in human. It might also indicate the persistence of intestinal parasitic infection in the area [7].

Conducting studies on the prevalence of intestinal parasites among vegetables and fruits in different parts of the country and identifying risk factors in the communities are important to design appropriate intervention strategies[36]. In the current study, *Ascaris lumbricoides* was found the most dominant parasite, with a prevalence of 24.4%. This finding has been supported by other researchers where *Ascaris lumbricoides* was the predominant parasite detected [37,38]. However, a lower rate of contamination of vegetables samples with *A. lumbricoides* eggs was recorded (1.8%) of total examined samples in Turkey [39]. Also, in Iran, as it was found to be 2.5% in Jiruft [40]. On other hand, our results disagreed with other studies that showed higher findings as in Zamfara States, Nigeria where *Ascaris* eggs were detected in 56.31% of the examined vegetables and fruits samples [41] and a very high percentage of *A. lumbricoides* (89.33%) was recorded by Abe *et al.* [42].

However, these differences between this study and other studies could be attributed to the difference in method used, health awareness, and living standards [43]. On the other hand, the predominance of *A. lumbricoides* may be due to the its worldwide distribution, the high number of eggs produced by the adult female parasite which contributes to the parasite ubiquitous distribution, and the resistant nature of the eggs that enables them to survive adverse conditions, since the eggs of *A. lumbricoides* can resist and survive for two years at 5–10 °C, and be unaffected by desiccation for two to three weeks [44]. In addition, the presence of *Ascaris* eggs in the vegetables could be attributed to the usage of untreated night soil [15].

In the present study, *Entamoeba histolytica/dispar* (20.9%) was the second most prevalent contaminant next to *Ascaris lumbricoides* (24.4%), and followed by *Entamoeba coli* (16.8%) and *Giardia intestinalis* (9.7%). Table (3).

This finding was in line with previous study conducted at two central markets in Khartoum, Sudan, where the most prevalent detected parasites in vegetable samples in both markets were *Entamoeba histolytica/dispar*, *Entamoeba coli*, and *Giardia intestinalis*[45]. Tefera *et al.*[46] in a study conducted in Jimma Town, Southwest Ethiopia, *Entamoeba histolytica*, *Entamoeba coli*, and *Giardia intestinalis* were considered to be the most common protozoan parasitic contaminants of vegetables and fruits. In addition, the presence of *Entamoeba histolytica*, and *Entamoeba coli* among the highest occurring parasites was found in other previous findings [35]. Moreover, our results were in agreement with the findings of a studies conducted by Tsegahun *et al.*, in Debre Berhan Town, Ethiopia, and by Alemu *et al.*, in Bahir Dar City, Northwest Ethiopia, where *Entamoeba histolytica* detected in 13.0% and 12.8% of examined vegetable samples, respectively [17,14]. However, in Gaza, Palestine, higher prevalence rate of 37.5% of vegetable samples contamination for *E. histolytica* was reported [47]. On the other hand, in Alexandria, Egypt, by Hassan *et al.* a low prevalence rate of 11.2% was recorded [48]. However, the difference in prevalence observed between studies might be due to difference in climatic conditions and geographical location [46, 49]. Detection of amoebae cysts indicates the possibility of the contamination of the vegetables by human feces since the organism only lives in the human intestine [35].

In the current study, *Entamoeba coli* was detected in 16.8 % of examined vegetables. Similar finding was reported in Tabuk, Saudi Arabia [50]. However, the high results obtained in this study could be explained by the use of iodine wet mount which increases the microscopical sensitivity for detection of protozoa cysts of *histolytica/dispar* and *Entamoeba coli*.

Giardia intestinalis cyst was detected in 9.7 % of examined vegetables. These findings were similar with previous studies, from Alexandria, Egypt where the prevalence of *G. intestinalis* cysts was 8.8% [48]. Another study in Libya reported that *G. intestinalis* cysts were found in 10% of the total examined vegetable samples [29]. However, high rates were reported from Asmara where the prevalence of *G. intestinalis* cyst was 19.7% of the examined samples of vegetables [51], and 23% of the examined samples of salad vegetables in Amman and Baqa'a in Jordan [52]. On other hand, low rates of 3.8% and 1.6% were recorded in a study performed by Al-Sanabani *et al.* [10] and Matini *et al.* [13]. This prevalence rate of *Giardia intestinalis* found in our study may be attributed to the long periods of survival of cyst form of *Giardia intestinalis* under moist environmental conditions and due to its resistance to many adverse conditions [53]. On the other hand, these parasites are usually associated with poor sanitary habits, lack of access to safe water and improper hygiene and the degree of each prevalence of parasites varies from one region to the other [54].

As seen in table 3, the major parasitic contaminants of parsley samples were *Ascaris lumbricoides* (17; 50.0%), followed by *Entamoeba histolytica/dispar* (17; 47.2%), *Entamoeba coli* (14; 83.9%), and *Giardia intestinalis* (8; 22.2%). This result was in accordance with a study in Hamadan, Iran [55], Syria [8], and in Iran [16] as parsley recorded with the highest level of contamination in fresh vegetable samples.

On parasitic contamination frequency of watercress samples, also *Ascaris lumbricoides* (16; 44.4%), *Entamoeba histolytica/dispar* (15; 41.7%), and *Entamoeba coli* (12;33.3%) were the major contaminants in watercress. These results were partially in accordance with a study in Sharkyia Governorate, Egypt, where the highest contaminated one was watercress (55.7%) followed by lettuce (45.7%) [15], and in Tripoli, Libya, where watercress and lettuce samples were found to be contaminated more than other samples [29]. The frequency of contamination of lettuce samples showed *A. lumbricoides* (14; 83.9%), followed by *Entamoeba histolytica/dispar* (10;27.8%), and *Entamoeba coli* (10;27.8%) were the dominants. Also, the present study was in assent with many previous studies [8, 56]. In leek the most commonly detected parasites was also, *A. lumbricoides* (12; 33.3%), *Entamoeba histolytica/dispar* (9;25.0%), and *Entamoeba coli* (8; 22.2%). Similar to our findings, leek was among the most infested vegetable in previous report [57] and in a study performed in Khorramabad, Iran, leek recorded with contamination rate of 80% [27]. The frequency of contamination of green onion sample showed dominances of *A. lumbricoides* (11;30.6%) and *Entamoeba histolytica/dispar* (8;22.2%). A similar findings were reported by previous studies [58]. In the present study, the least

number of parasites were found on tomato. These results were in accordance with studies which reported that green leafy vegetables such as parsley, watercress, and lettuce have uneven surfaces that probably facilitate sticking of parasitic eggs, cysts, and oocysts more readily, either at the farm or when washed with contaminated water. On the other hand, vegetables with smooth surface such as leek, green onion, and tomatoes, had the lowest contamination because its smooth surface reduces the rate of parasitic attachment [59, 60, 38]. However, difference in the level of contamination and parasitic species involved in contamination of examined vegetables vary from place to place due to variations in environmental and human factors associated with its production, transport, handling, and processing in the different study areas [61]. Also, the high level of contamination and multiple species contamination observed among examined vegetables in the current study, might indicate the persistence of intestinal parasitic infection in the area [38].

In this study, even though it was statistically insignificant, the contamination rate was different for the samples collected from different markets. In this study, vegetable samples collected from Dar Saad market showed a higher rate (94.4%) of contamination, while the lowest contamination rate (50.0%) was among vegetables collected from Al Mansura market. Table (4). Al Mansura market is considered as the main wholesale market to which vegetables and fruits are transported by farmers, traders and wholesalers from different farms and agricultural areas in different parts of Yemen. From there, vegetables and fruits are transported and distributed to the other markets and retailers in Aden governorate.

The higher contamination rates of vegetables collected from the studied markets could be attributed to many different factors including the transportation of vegetables and fruits. The transportation of vegetables and fruits from Al Mansura market to the other markets usually occurs by motor-cycles, tri-cycles or in dirty vans or bus which sometimes are loaded with passengers. This indicate that socio-cultural practices in certain geographical areas as the main causes of different levels of contamination [62]. Another contributing factor in contamination of vegetables could be the lack healthy piped water. On observation, the water was brought from outside the markets, and carried by water tankers or dirty containers and most of the water samples used to wash and sprinkle vegetables were dirty due to dust and vegetable debris. This findings supported by a study in which there was 14% of 50 water samples used to sprinkle vegetables were brought from outside the market in dirty containers and carried by cart as the markets lack healthy piped water [63].

One more contributing factor in contamination of vegetables could be the poor hygienic practices. In these

markets there is unhygienic handling of vegetables by vendors who display their vegetables on the floor as observed during the purchasing, which may have exposed the vegetables to direct contact with the soil and contaminants. This is in consonance with Edosomwan and his colleagues [64] who reported higher contamination rates in markets where the vegetables were placed on the floor which might have been contaminated with different parasitic forms such as eggs or cysts. Therefore, the high contamination rates reported in the selected markets could be explained by the act of washing produce before display and by the way the products are displayed [65]. Furthermore, the high contamination level reported in the vegetables collected from the selected local markets in this study could be also attributed to varying environmental conditions such as poor drainage systems [66,67]. Moreover, in Aden governorate, majority of markets are open-aired markets and some are partially closed. Therefore, the appropriate local public health authority is recommended to establish a system for continuous monitoring of contamination of vegetables and fruits sold at local markets and a comprehensive health education should be given to vendors of vegetables and to the general population on the health risks associated with consumption of contaminated vegetables [68].

However, the different rate of contamination found in different markets might be due to the different sources of vegetables as reported by other researchers [30, 69]. On the other hand, the low level of contamination rate of 50.0% recorded in vegetables collected from Al Mansura market, might be due to that Al Mansura market is a wholesale market, while the others are retail markets, in which produces subjected to more processing and handling i.e. more contamination.

Conclusion and Recommendation

This study highlighted the importance of raw vegetables as the potential source of transmission for intestinal parasites to humans. The consumers should always observe the basic principle of food and personal hygiene, that is, thorough washing of the vegetables before eating and washing hands before meal. A comprehensive health education should be given to vendors of vegetables and to the general population on the health risks associated with consumption of contaminated vegetables. The local public health sector should establish a system for continuous monitoring of contamination of vegetables sold at local markets.

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التلوث الطفيلي للخضار في الأسواق المحلية المختارة في محافظة عدن، اليمن

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المُلخَص

على الرغم من ان الخضروات الطازجة تلعب دور مهم في تزويد جسم الإنسان بمختلف المواد الأساسية والضرورية إلا أنه عند تلوثها يمكن ان تنتقل الأمراض المعوية الطفيلية. وقد هدفت هذه الدراسة الى تحديد مستوى التلوث الطفيلي للخضروات التي يتم تناولها بشكل نبيء في محافظة عدن، اليمن. تم أجرى دراسة مقطعية تحليلية وصفية لتقييم مستوى التلوث الطفيلي للخضروات التي تباع في ست أسواق محلية مختارة في محافظة عدن. حيث تم جمع 216 عينة تتكون من ستة أنواع مختلفة من الخضروات وهي البقدونس (*Petroselinum sativum*)، الجرجير (*Nasturtium officinale*)، الخس (*Lactuca sativa*)، الكراث (*Allium porrum*)، البصل الأخضر (*Allium cepa*) والطماطم (*Solanum Lycopersicum*) تم شراؤها خلال الفترة من يوليو حتى ديسمبر 2022م. تم أخذ 250 جرام من كل عينة و فحصها باستخدام طريقة الترسيب البسيط وطريقة التعويم باستخدام كبريتات الزنك و تم تحضير الشرائح باستخدام التحضير الرطب واليود ومع صبغة تسيل - نلسن (Ziehl- Neelsen) و تم الفحص بواسطة المجهر الضوئي. أظهرت الدراسة تلوث 166 عينة (76.9%) بطفيل واحد أو أكثر. وكان البقدونس الأكثر تلوث (97.2%)، يليه الجرجير (94.4%)، ثم الخس (88.9%) وكان الطماطم الأقل تلوث (22.2%). وجد ان هناك فوارق ذات دلالة معنوية بين معدلات التلوث وأنواع الخضروات. وكانت بيوض دودة الأسكارس 75 (24.4%) الأكثر شيوعا، تليها المتحولة الحالة للنسج والديسبارية 64 (20.9%) ثم المتحولة القولونية 52 (16.8%). وجد ان هناك فروق ذات دلالة معنوية بين نسب الانتشار لأنواع المختلفة من الطفيليات. أظهرت الدراسة بأن الخضروات المشتراة من سوق دار سعد كانت الأكثر تلوث (94.4%) والمشتراة من سوق المنصورة هي الأقل تلوث (50.0%). لم تكن هناك فروق ذات دلالة معنوية بين معدلات التلوث بين الأسواق. أظهرت الدراسة أهمية الخضار كمصدر محتمل لأنتقال الطفيليات المعوية الى الإنسان. ولذلك يجب ابلاغ المستهلكين على ضرورة غسل اليدين بشكل جيد وغسل وطهي الخضروات قبل تناولها بالإضافة الى ضرورة التنقيف الصحي ونشر الممارسات الصحية بين أوساط الباعة والمستهلكين.

الكلمات المفتاحية: الخضروات، التلوث، الطفيليات المعوية، الأسواق.

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