

Detection of Human Intestinal Parasites in Commonly Consumed Fresh Leafy Vegetables in Al-Baha Region, Saudi Arabia

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Abstract

Introduction: Ingestion of contaminated fresh leafy vegetables had an important role in the transmission of several intestinal parasitic diseases.

Objectives: This study aimed to identify human intestinal parasites in commonly consumed fresh leafy vegetables in Al-Baha Region, Saudi Arabia.

Methodology: In the current study, 400 leafy vegetable samples collected randomly from the supermarkets and open-aired markets at different times of the year in Al-Baha city were examined to detect the frequency of contamination with human intestinal parasites. Sediments and supernatants of concentrated washing solution of vegetables were examined after stained by iodine and modified Ziehl-Neelsen.

Results: The leafy vegetables contaminated with intestinal parasites were 41% (164/400). The most contaminated leafy vegetables were spinach, red radish, watercress and lettuce with 58.3%, 57.5%, 55% and 50%, respectively, while the less contaminated were mint and cabbage with 16.6% per each. Green onions, parsley, dill, celery, leek and coriander were contaminated with 40%, 37.5%, 33.3%, 31.2%, 25% and 21.9%, respectively. Contaminated leafy vegetables collected from open-air markets was 41% compared to 33% of that were collected from supermarkets. These results were statistically significant. About 122 leafy vegetables out of 164 (74.3%) were contaminated with helminths. Taeniid eggs and *Strongyloides* larvae were the most common with 18.3% (30 leafy vegetables per each). About 141 leafy vegetables out of 164 (85.9%) were contaminated with pathogenic human intestinal protozoa. *Cryptosporidium* oocysts and *Giardia lamblia* cysts were the most commonly found with 48 (29.3%) and 35 (21.3%), respectively. The highest prevalence was in spring (45.5%) and the lowest was in summer (33.3%). These results were statistically significant.

Conclusion: Control measures should include treatment of irrigation water, municipal wastewater, treatment of infected persons and mass education of the persons in contact with fresh leafy vegetables to avoid a greater health risk from handling and consuming these vegetables.

Keywords: Human Intestinal Parasites; Fresh Leafy Vegetables; Al-Baha Region; Foodborne Disease (FBD)

Introduction

Diarrhea is a common clinical manifestation of foodborne disease (FBD) that is an increasing public health problem and responsible for considerable morbidity and mortality globally [1-3]. Many factors are affecting food safety, such as contamination of water and food, inappropriate storage and food transportation and unhygienic food practices therefore, FBDs are more prevalent in developing countries [4]. Food prepared and consumed at home is a source for a large proportion of foodborne diseases where consumers do not follow proper food handling practices [5-8]. Foodborne illnesses linked to consuming and handling of contaminated fresh vegetables represents one of the major forms of transmitted parasites into the human [9-11]. Vegetables can be contaminated during growth due to sewage sludge, untreated wastewater, contaminated slurry, farm livestock and indigenous [12,13]. Also, wild and domestic animals may be included as sources of contamination [13]. Parasites that are detected in leafy vegetables collected from open-aired markets have a marked difference in prevalence rates when compared with that collected from supermarkets [13,14]. The transmission of these parasites to the human occurs through ingestion of the parasite environmental transmission stages such as cyst or oocysts in protozoa and egg and larvae on helminths [14]. In humans, multiple factors are controlling the outcomes of parasitic infection such as the species, genotype of the parasite, infective dose and life stage of the parasite, host immunity, infection site and incubation period [15]. The clinical illness may be mild to severe and manifested within days to weeks, or it may appear as a long-term disease depending on these previous factors [16].

Several surveys from different parts of the world showed that vegetables can be an agent for the transmission of protozoan cysts and oocysts such as *Giardia*, *Cryptosporidium*, *Entamoeba*, *Toxoplasma* and *Cystoisospora*. Besides, helminths eggs such as *Taeniid*, *Ascaris*, *Hymenolepis*, *Fasciola*, *Toxocara* and larvae of *Strongyloides*, *Trichostrongylus* and *Hookworms* were reported [12-14]. In developing countries, routine diagnosis for monitoring foodborne pathogens is inadequate and nonexistent so, most outbreaks caused by contaminations of fresh leafy vegetables go undetected [16]. In Saudi Arabia, Al-Beinali and his co-workers found that a high rate of parasitic infection among raw vegetables collected from Abhaa province in the southwestern of the kingdom [17]. Whereas Al-Megrin found different rates of intestinal human parasites in raw vegetables collected from the Riyadh sector [18]. The difference in climates in Saudi sectors maybe has an important role in the fre-

quencies and types of parasitic contamination of vegetables. Higher rates of contamination were reported in the warm season than those during the cold season and in the dry season than the rainy season [17,18]. Al Baha area has different weather characterized by low temperature than other areas in the Saudi Arabia Kingdom [19]. The region of Al Baha is also home to several free-range animals, such as baboons, donkeys, dogs, cats and many birds and rodents. At the same time, it is known for the fertile lands, which are used for the production of many fruits and vegetables [20].

Aim of the Study

This study aimed to determine the degree of contamination caused by human parasites in commonly used leafy vegetables collected from Al-Baha, Saudi Arabia and its relation to the season.

Materials and Methods

Description of the study area: Generally, the climate in Al-Baha is mild with temperatures ranging between 12 to 23°C (53.6 to 73.4°F). Due to its location at 2,500 meters (8,200 ft) above sea level, Al Baha's climate is moderate in summer and cold in winter. The area attracts visitors looking for a moderate climate and pristine. Its province is known for forests, wildlife areas, valleys and mountains. Humidity ranges from 52% - 67%. While in the mountainous region, which is known as As-Sarah, the weather is cooler in summer and winter. Rainfall in the mountainous region lies in the range of 229 to 581 millimeters (9 to 23 in). The average throughout the whole region is 100 to 250 millimeters (3.9 to 9.8 in) annually [19].

Samples: In the current study, 400 leafy vegetable samples were collected randomly from the supermarkets and open-aired markets from the following: Spinach (50), dill (50), lettuce (40), parsley (40), watercress (40), green onion (40), red radish (40), leek (32), coriander (32), celery (16), cabbage (12) and mint (12). The study was conducted at different times of the year (autumn, winter, spring and summer) in Al-Baha city. The samples were obtained from Al-Baha Open aired markets and supermarkets (200 samples per each). Each fresh sample was put in a separate nylon bag and labeled with a unique number and date of collection. The collection of samples occurred from September 2018 to August 2019. The samples were processed within 1 - 10 hours of collection.

Methods: According to Al-Megrin [16] and Abougrain., *et al.* [21], a subset from each sample (100 - 150 mg) was chopped into small pieces and soaked in a plastic bag containing one liter of physi-

ological saline solution for 15 minutes. Then the plastic bag with vigorous shaking with the aid of a mechanical shaker for 15 minutes. The vegetable sample was removed and the remaining washing solution was left overnight to sediment. Then, the top layer was discarded (approximately 800 ml) and the remaining wash solution was filtered through a sieve with 425 um pore size to remove large debris and the remainder well transformed to four tubes (each was 50 ml in size) and centrifuged at 2000g for min. Following centrifugation, the supernatant was transported into another two test tubes to be floated, separately, by both Zinc sulfate and Sheather’s sugar [22]. Then, three drops of floats were examined, one directly, the second was stained with Lugol’s iodine and the last drop was stained with modified Ziehl-Neelsen. After the supernatant was removed from the concentrated sample, the sediment was examined under a light microscope with and without staining (Lugol’s iodine and modified Ziehl-Neelsen). From each sample, three slides/each tube were prepared for each diagnostic method. Data will be collected and analyzed according to the total prevalence and prevalence/each parasite, prevalence according to the site of sold and according to prevalence seasonal variation.

Statistical analysis

A Chi-square test was used for statistical analysis. The level of significance for p values was accepted at $p \leq 0.05$.

Results

In the present study, the frequencies of contaminated leafy vegetables with human intestinal parasites were 164 out of 400 with 41% (Table 1). The most contaminated leafy vegetables were spinach, red radish, watercress and lettuce with 58.3%, 57.5%, 55% and 50%, respectively, while the less contaminated were mint and cabbage with 16.6% per each. Green onions, parsley, dill, celery, leek and coriander were contaminated with 40%, 37.5%, 33.3%, 31.2%, 25% and 21.9%, respectively. In the current study, the prevalence of parasite contamination among leafy vegetables collected from open-air markets was 41% compared to 33% of that collected from supermarkets (Table 2). These results were statistically significant. Regarding to the types of vegetables and markets only spinach and lettuce showed statistically significant.

The distribution of the intestinal parasites, 122 leafy vegetables out of 164 (74.3%) were contaminated with helminths. *Taeniid*

Type of examined vegetables	Number of examined	Non-contaminated		Contaminated	
		No	%	No	%
Spinach	48	20	41.7	28	58.3
Dill	48	32	66.7	16	33.3
Lettuce	40	20	50	20	50
Parsley	40	25	62.5	15	37.5
Watercress	40	18	45	22	55
green onion	40	24	60	16	40
Radish	40	17	42.5	23	57.5
Leek	32	24	75	8	25
Coriander	32	25	78.1	7	21.9
Celery	16	11	68.8	5	31.2
Cabbage	12	10	83.3	2	16.6
Mint	12	10	83.3	2	16.6
Total	400	236	59	164	41

Table 1: The contamination percentages of leafy vegetables by enteric human parasitic stages in Al-Baha.

eggs and Strongyloides larvae were the most common parasites with 30 leafy vegetables per each (18.3%) and the less frequent were hookworms’ eggs with 3 vegetable samples (2.6%) followed by four samples contaminated with *Trichostrongylus* larvae (Table 3). *Ascaris* and *Toxocara* eggs were detected in 14.6% and 12.2% of the contaminated plant, respectively, while *Trichuris* and *H. nana* eggs were identified in 3.7% and 3.1%, respectively. About 141 leafy vegetables out of 164 (85.9%) were contaminated with human enteric protozoa. *Cryptosporidium* oocysts and *Giardia* cysts were the most commonly found with 48 (29.3%) and 35 (21.3%) of leafy vegetables and the less frequent were *Balantidium coli* cysts and *Cystoisospora* with 2 (1.2%) and 3 (1.8%), respectively (Table 4). *Cyclospora*, *Entamoeba*, *Blastocystis* and *Toxoplasma* oocyst were demonstrated in 15.9%, 6.7%, 6.7% and 3%, respectively.

In the present study, a statistical significant seasonal variation in the relations to the prevalence of parasitic contamination of leafy vegetables was detected whereas the highest rate was found in summer (66.7%) and lowest one was in winter (20%).

Type of examined vegetables	Supermarkets		Open Aired Markets		Contaminated		Chi-square/Fisher exact test p-value
	No	%	No	%	No	%	
Spinach	10	20.8	18	37.5	28	58.3	.019172*
Dill	6	12.5	10	20.8	16	33.3	.220671
Lettuce	6	15	14	35	20	50	.011412*
Parsley	8	20	7	17.5	15	37.5	.743971
Watercress	10	25	12	30	22	55	.52501
Green onion	5	12.5	11	27.5	16	40	.052808
Red Radish	9	22.5	14	35	23	57.5	.109769
Leek	4	20	4	30	8	25	1
Coriander	4	12.5	3	9.4	7	21.9	.668929
Celery	2	12.5	3	18.7	5	31.2	.589639
Cabbage	1	8.3	1	8.3	2	16.6	1
Mint	1	8.3	1	8.3	2	16.6	1
Total	66	33	98	49	164	41%	.001141*

Table 2: The contamination percentages of leafy vegetables by enteric parasitic stages of different markets in Al-Baha.

*: The difference is statistically significant.

Type of contaminated vegetables	Taeniid eggs		H nana eggs		Ascaris eggs		Trichuris eggs		Toxocara eggs		Hookworms eggs		Strongyloides Larvae		Trichostrongylus Larvae		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Spinach 28	2	7.1	0	0	6	21.5	0	0	2	7.1	0	0	10	35.7	0	0	20	71.4
Dill 16	2	12.2	1	6.2	0	0	2	12.2	3	18.6	1	6.2	0	0	1	6.2	10	62.5
Lettuce 20	3	15	1	5	4	20	1	5	1	5	0	0	1	5	1	5	12	60
Parsley 15	5	33.3	1	6.7	1	6.7	1	6.7	2	13.3	1	6.7	0	0	0	0	11	73.4
Watercress 22	5	22.7	1	4.8	4	19.2	1	4.8	2	9.6	1	4.8	2	9.6	0	0	16	72.7
G. Onion 16	4	25	0	0	2	12.5	0	0	3	18.6	0	0	3	18.6	1	6.6	13	81.3
Red Radish 23	3	13	1	4.3	4	17.2	0	0	3	13	0	0	7	30.6	0	0	18	78.2
Leek 8	1	12.5	0	0	2	25	0	0	0	0	0	0	2	25	1	12.5	6	75
Coriander 7	2	28.6	0	0	0	0	1	14.2	2	28.6	0	0	2	28.6	0	0	7	100
Celery 5	1	20	0	0	1	20	0	0	0	0	0	0	3	60	0	0	5	100
Cabbage 2	1	50	0	0	0	0	0	0	1	50	0	0	0	0	0	0	2	100
Mint 2	1	50	0	0	0	20	0	0	1	50	0	0	0	0	0	0	2	100
Total	30	18.3	5	3.1	24	14.6	6	3.7	20	12.2	3	1.8	30	18.3	4	2.4	122	74.4

Table 3: Distribution of the pathogenic intestinal helminths among leafy vegetables collected from in Al-Baha.

Type and numbers of contaminated vegetables	Entamoeba cyst		Giardia cyst		Balantidium cyst		Blastocystis cyst		Toxoplasma oocyst		Cryptosporidium oocyst		Cyclospora oocyst		Cystoisospora oocysts		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Spinach	2	7.1	2	7.1	1	3.6	2	7.1	1	3.6	10	35.7	4	14.4	0	0	22	78.6
Dill	2	12.6	2	12.6	0	0	2	12.6	1	6.3	3	18.8	2	12.6	1	6.3	15	93.8
Lettuce	0	0	2	10	0	0	0	0	0	0	4	20	10	50	0	0	16	80
Parsley	0	0	4	26.7	0	0	1	6.6	0	0	4	26.7	4	26.7	1	6.6	14	93.3
Watercress	2	9	6	27	0	0	1	4.5	1	4.5	8	36	2	9	0	0	20	91
Green Onion	1	6.3	5	31.1	1	6.3	1	6.3	1	6.3	4	25	0	0	0	0	13	81.3
Red Radish	3	13	4	17.4	0	0	2	8.8	0	0	9	39.3	1	4.4	0	0	19	73.9
Leek	0	0	3	37.5	0	0	1	12.5	0	0	4	50	0	0	0	0	8	100
Coriander	0	0	6	85.7	0	0	1	14.3	0	0	0	0	0	0	0	0	7	100
Celery	1	20	0	0	0	0	0	0	0	0	0	0	3	60	1	20	5	100
Cabbage	0	0	1	50	0	0	0	0	0	0	1	50	0	0	0	0	2	100
Mint	0	0	0	0	0	0	0	0	1	50	1	50	0	0	0	0	2	100
Total	11	6.7	35	21.3	2	1.2	11	6.7	5	3.0	48	29.3	26	15.9	3	1.8	141	85.9

Table 4: Distribution of the pathogenic human intestinal protozoa among leafy vegetables collected from in Al-Baha.

Seasons	Contaminated		Not-Contaminated		Total		p-value
	No	%	No	%	No	%	
Autumn	44	44	56	56	100	100	.00001
Winter	20	20	80	80	100	100	
Spring	40	36.4	70	63.6	110	100	
Summer	60	66.7	30	33.3	90	100	
Total	164	41	236	59	400	100	

Table 5: The frequency of Contaminated leafy vegetables compared to not-contaminated with the seasonal distribution. The result is statistically significant.

Discussion

The force of habits of eating fresh leafy vegetables particularly as an essential component of salad plays a critical epidemiologic role in transmitting parasitic food-borne diseases in the presence of the infective stages through handling and consuming [14].

Results from this study demonstrated that 41% of the fresh leafy vegetables collected from Al-Baha were contaminate with human enteric parasites (Table 1). Similar reports demonstrated the frequency of parasitic contamination in some parts of Saudi Arabia but notably it was high in our study. In agreement with our results, Gabber and Shaker estimated an overall prevalence rate of 46% (184 out 400) of parasitic contamination among eight leafy veg-

etables in Tabuk [23]. Also, Albeinali and his co-workers found that a high rate of parasitic infection among 5 raw vegetables collected from the central vegetable market in Abhaa city in the southwestern of the kingdom with 27.2% [17]. In controversy, a low prevalence was reported on Riyadh that only 76 out of 470 samples (16%) were contained parasite stages [18]. In Al Qassim, the prevalence rate ranged from 23.8% to 36.9% for contaminated with human intestinal helminths and protozoa [24]. When raw vegetables collected from cities of the Sarawat Mountain of Saudi Arabia including lettuce, green onions, cucumbers, carrots, spinach, tomatoes, parsley, ginger, radish and arugula were examined to identifying the frequency of parasitic contamination the rates were 19% (38/200) in Albaha, followed by 15% (30/200) in Baljurish, 12.5%

(25/200) in Altaif and 11% (22/200) in Abha [19]. Globally regarding some developing countries, the prevalence of contamination was high in Kenya and in Libya with 75.6% and 58%, respectively while it was lower than our results with 31.7% and 29% in Egypt and Iraq, respectively [25-28]. Therefore, the difference between our study results and the other study were referred to the weather variability, types of vegetables, sample size/each, seasons of collection, vegetable and the methods used in the examination.

In the current study, the prevalence of parasite contamination among leafy vegetables collected from open-air markets were 41% compared to 33% of that collected from supermarkets. These results were statistically significant although no statistically significant results were detected per certain type of vegetable particularly. In agreement with our results intestinal human parasites that are detected in leafy vegetables collected from open-aired markets have a marked difference in prevalence rates when compared with that collected from supermarkets [13,14]. This may be referred to the degrees of contamination through handling the vegetables that are less in the supermarket than in open-aired markets. The most contaminated leafy vegetables in our study were spinach and red radish with 60% and 59.3%, respectively, while the less contaminated were mint and cabbage with 14.3% and 15%, respectively (Table 2). Green onions and Coriander were contaminated with 41.2% each. Parsley, leek and lettuce were contaminated by 40% each. Watercress and dill were contaminated by 37.5% and 36%, respectively. Al Megrin demonstrated that the rates of parasitic contaminations were 27.8% (17/61) in lettuce, 22.8% (13/57) in watercress, 20.6% (7/34) in leek, 19.1% (9/47) in green onion, 17.4% (15/87) in parsley, 15.4% (4/26) in spinach, 13.6% (3/22) in basil, 11.5% (3/26) in coriander, 9.4% (3/32) in radish, 5.3% (1/19) in dill and 4.7% (2/42) in mint. No parasites were detected in 17 samples collected from cabbage [18]. Albinali, *et al.* found that in descending order 28% of green onion, 25% of reddish, 17% of watercress, 17% lettuce and 13% of leek were contaminated [17]. The prevalence in Tabuk was 15.76% in cabbage and 14.13% in watercress, 13.04% in lettuce and 8.7% in green onion [23]. In others developing countries such as Egypt they supported our results as the high rate of contamination varies according to the shape and surface of vegetables whereas watercress, lettuce and parsley were the most contaminate [28,29]. Also, lettuce and water cress were contaminating significantly than other vegetables in Libya [25]. The variability in frequencies may be related to the number and type of examined vegetables as previously mentioned in the study

of Alqumber [19] who demonstrated that the highest contamination was observed in radishes, parsley, carrots, spinach and ginger while the lowest contamination was detected in cucumbers, tomatoes and green onions. Furthermore, in a study was done in Riyadh, women were at risk of improper food -holding temperature with 45.28%, cross contaminations were 32.23% and food from unsafe sources was 22.39% [30]. In additions, some vegetables are highly susceptible to infection through post harvesting handling methods such as spinach and red radish which are the most contaminated vegetable in our study.

Regarding the distribution of the intestinal parasitic helminths among leafy vegetables collected from in Al-Baha, 122 leafy vegetables out of 164 (74.3%) were contaminated with helminths. In the present study, *Taeniid* eggs detected in 18.3% of leafy vegetables that may be belonged to *Echinococcus granulosus*, *Taenia spp.* and *Multiceps multiceps*. They cause hydatidosis, coenouris and neurocysticercosis (*Taenia solium* infection) that were showed in KSA previously [17]. A high frequency of *Taeniid* eggs was coordinated with a study done in Riyadh with a prevalence rate of 19.7% [18]. In contrast, low prevalence's were showed in al-Qassim and Tabuk with 10% and 4.35%, respectively [23,24]. Also, no *Taeniid* eggs were recorded in Abha [17]. The zoonotic transmission of *Taeniid* may have a role in the high rate in our study. These can be explained in the case of *Taenia solium* as it should be of human origin where many of the expertise are non-Muslims. Also, *Strongyloides* larvae were detected in 18.3% of contaminated leafy vegetables in the present study. In agreement with our results, Omar, *et al.* demonstrated a 15% contamination of leafy vegetables collected from Hail [31]. Low prevalence was showed in Tabuk with 9.24% [23]. The contamination by this parasite was zero among leafy vegetables collected from both Al-Qassim, Riyadh and Abha [17,18,24]. Although *Strongyloides* infection in human span from asymptomatic light infections to chronic symptomatic but hyperinfection syndrome and a potentially fatal spread of the larvae in immunocompromised patients could lead to 85% mortality rate [32]. In the current study, *Ascaris* and *Toxocara* eggs were detected in 14.6% and 12.2% of the contaminated plant, respectively, while *Trichuris* and *H. nana* eggs were identified in 3.7% and 3.1% respectively. The less frequent were hookworms' eggs with 1.8% and *Trichostrongylus* larvae (2.6%). In agreement with our data Albinali, *et al.* demonstrated that *Ascaris lumbricoides* was detected with 15.8% while in contrast to our results *Ancylostoma duodenale* was 19% [17]. Moreover, *Ascaris* demonstrated with

21.74% in Tabuk. In contrast in Riyadh spp of *Ascaris*, *Hymenolepis* and *Ancylostoma* was demonstrated in high prevalence rates than our study with 26.3%, 14.5% and 11.8%, respectively, while in the case of *Trichostrongylus* (2.6%) the rate is similar to our result [18]. In another study, *Trichuris trichiura* and *Trichostrongylus* were the most commonly detected infectious agents in the raw vegetables, while *Hymenolepis nana* was the least prevalent [19]. The zoonotic transmission particularly from stray cats, dogs and primates may be play a role in the high prevalence of helminths eggs in our study.

In the present study, the distribution of the intestinal parasitic protozoa 41 leafy vegetables out of 164 (85.9%) was contaminated. *Cryptosporidium* oocysts and *Giardia* cysts were the most commonly found with 48 (29.3%) and 35 (21.3%) leafy vegetables and the less frequent were *Balantidium coli* cysts and isopora oocysts with 1.2% and 1.8%, respectively (Table 4). *Cyclospora* oocysts, *Entamoeba* cysts, *Blastocystis* cysts and *Toxoplasma* oocysts were demonstrated in 15.9%, 6.7%, 6.7% and 3%, respectively. In agreement with the current study results, Al-Migrin showed that *Giardia lamblia*, *Blastocystis* and *Toxoplasma gondii* were detected in 31.6%, 17.1% and 6.6%, respectively [18]. Besides, *Entamoeba* sp was the common protozoa in contaminated vegetables collected from Tabuk while *Giardia* was only showed in 2.17% [23]. Our results were in controversy with Albainali, *et al.* who demonstrated that *Blastocystis* (12.6%) were the most common isolated protozoa [17]. In Al-Qassim, *Giardia* were 20.7%, *Cryptosporidium* were 13%, *Entamoeba* were 10.7% and *Balantidium coli* were 2.3% [22]. In the other side, no protozoa were detected in the study of Alqumber [19]. This variability may be related to the role of zoonotic contamination as there are many domestic and wild animals present in Al-Bahaa. In addition, *Cryptosporidium* oocysts had the ability to strongly adhering to spinach and were internalized by the leaves therefore it was the most detected parasites in some studies [29]. In Spain 12 out of 19 (63.2%) samples of green vegetables used in salads products were contaminated with cryptosporidium only [32]. However, *Giardia* is the most prevalent parasite in contaminated leafy vegetables in another study [30]. The use of sewage water for irrigation is a common way of these parasitic contaminations. For *Cryptosporidium Spp.* and *Giardia lamblia*, a large range of hosts may be infected with shedding oocysts/cysts in their faeces that can resist the variabilities in weather easily [33,34]. Dose-response modelling predicts a probability of human infection following ingestion of a single oocyst of *Cryptosporidium* could be as high as 72% [35] while the minimal infective dose of *Giardia* is less than 10 cysts [34].

Regarding seasonal variation, the present study results indicated a statistically significant seasonal variation, with high rate in summer (66.7%) followed in autumn (44%), springer (36.4%) and winter (20%). Our results are consistent with previous studies that reported higher rates of parasitic contamination in vegetables during warm seasons than cold talking in consideration weather of summer in Al-Baha likes weather of springer in other areas. In contrast, the parasites were more common from September to December (autumn) in Abha [17]. In the study of Riyadh, the highest prevalence was showed in spring (23.1%), followed in descending order by summer (17.9%), autumn (10.6%) and winter (9.9%) [18]. These can be explained by the excretion of parasites resistance stages from human or animals to the environment are more common in warm weather than cold [25]. Also, post-harvest fecal contamination of vegetables may occur during handling and transport of vegetables and this may occur during splashing the vegetables with contaminated water or unhygienic hands to keep them fresh and by this way the parasite transmission to the human becomes easy [25]. In Egypt the rate of parasitic contamination in vegetable samples was the highest in summer (49%) and the lowest was in winter (10.8%) [29]. The excretion of parasite's eggs to environment by human or animals is high in warm seasons compared to cold seasons [37]. Another study in Hanoi, Vietnam, showed that the number of eggs recovered from vegetables was higher in the dry season (78%) than in the rainy season (22%) and it is assumed that eggs on the surface of vegetables are washed away by rain [38].

The high level of vegetable contamination with parasitic stages in this study is significant hence control measures should include treatment of irrigation water, municipal wastewater before use, treatment of infected persons and mass education of the populace on the danger of eating inadequately washed or raw vegetables. Provision of a good sanitary system in rural and urban areas should be adopted.

Conclusion

Control measures should include treatment of irrigation water, municipal wastewater, treatment of infected persons and mass education of the persons in contact with fresh leafy vegetables to avoid a greater health risk from handling and consuming these vegetables.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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