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SOIL-TRASMITTED PARASITES IN VEGETABLES IRRIGATED WITH WASTE-WATER IN FAYOUM GOVERNORATE, EGYPT

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Abstract

No doubt, irrigation with waste water became a painful reality in villages in Fayoum Governorate mostly because of water scarcity and improper sewage disposal. Ingestion of raw vegetables particularly when fresh imposes a great risk on population. Protozoa cysts as *Entamoeba, Giardia, Cryptosporidium, Cyclospora* and helminthic eggs can readily be transmitted by contaminated water or vegetables.

The study evaluated the contamination and infectivity of raw fresh vegetables in some Fayoum Governorate villages, which used waste water for irrigation

This cross-sectional study microscopically evaluated 360 fresh vegetable samples from different areas in for parasitic infective stages and potential infectivity. Also, 400 native patients suffered from gastrointestinal manifestations were examined for parasitic infection(s). Morning stool samples were microscopically examined for parasitosis. direct microscopy, concentration methods, staining with trichrome and modified ZN stain, Rapid immune-chromatographic assay for *Cryptosporidium*, *Giardia* end *Entamoeba* spp. are done.

The results showed that increased prevalence of parasitic contamination in villages of Fayoum Governorate, due to notorious of water shortage and use of untreated sewage water for irrigation, also there is corresponding increase in parasitic infection among population of the same locations.

Key words: Fayoum, Immunochromatographic, Irrigation, Parasitic, Sewage, Staining.

Introduction

Water is considered the most important source of life, and many countries face challenges in providing safe water for drinking and agriculture. Egypt is facing many challenges in this field, the most important of which is the continued decrease per capita share of water over the past years it reached less than 570m^3/ year noting that the limit of water scarcity is estimated at 1000m^3/capita /year (FAO, 2017) and this shortage will reach its climax after the operation of the Ethiopian Renaissance Dam which poses a great threat on Sudan and Egypt's water share, and lead many countries to treat wastewater in agriculture, but this process is facing many challenges being complicated and very expensive in a developing countries (Salgot *et al,* 2018).

Many farmers throughout the country use polluted sewage water to grow crops, in addition to the lack of a good sanitation network in many areas, and some citizens resort to draining sewage water into adjacent water streams, in 2016 some countries banned Egyptian exports from certain types of vegetables and fruits after some of their citizens had suffered from Hepatitis A after consuming some fruits imported from Egypt (Aboubakr and Goyal, 2019).

Shortage of water affecting many agricultural areas in Egypt has forced farmers to resort to using untreated sewage water, despite containing high levels of harmful chemical from animal and human waste that over time will pose major health risks to consumers.

About 90% of the rural houses in areas lack sanitary sewers, with illegally dispose of excreta and waste water to the nearby canals (Zahra and Rsheed, 2017).

Intestinal parasites and diarrhea cause great impact of human health mainly children as the second cause of death with more than 20% deaths annually (WHO, 2017). No doubt, consumption of raw vegetables and fruits play an important role in transmitting parasites to humans in many developing and developed countries (Yusof *et al,* 2017).

Fayoum is an agricultural governorate suffered also from water scarcity in many places, water complaints from farmers had arisen which forced them to use sewage water being the only way for irrigation.

Moreover, in recent years, Fayoum governorate has witnessed a decline in human development and service projects. The village of Sinro in Fayoum governorate of Ibshway Center is one of the poorest villages followed by the village of Sinhour in the center of Snores, more than 60% of the population of these villages are infected with viral and hepatic diseases, caused by the lack of both drinking and irrigation water. Also, El-Khamseen village in central Etsa was classified among the Egyptian poorest and neediest villages. These places are notorious by using sewage water in irrigation.

The study aimed to evaluate parasitic contamination and infectivity of raw vegetables that eaten in 4 different villages in Fayoum Governorate

Materials and Methods

A cross-sectional study was done from January to December 2019 The 360 samples of six types of eaten raw, green leafy vegetable were 60 samples from each of lettuce (*Lactuca sativa*), arugula (*Eruca sativa),* green onion (*Allium ascalonicum*), leek (*Allium porrum*), parsley (*Petroselinum hortense*), & radish (*Raphanus sativus*). Samples were purchased fresh from different markets and street vendor in the early morning. One hundred samples were from each village of Sinro, Ibshway Center, Sinhour, Snores Center, El-Khamseen, Etsa Center, and only 60 from Fayoum local markets

Vegetables preparation: About 150grams of each vegetable were prepared from the edible part. Samples were soaked in 100ml formol saline in a sealed plastic bag and agitated several times then transferred to a conical glass container. They were allowed to stand overnight for sedimentation. Supernatant was discarded leaving about 10-15ml residue centrifuged for 5min. at 4,000rpm, and microscopically examined by Lugol's iodine, and modified ZN stain for infective stages (Cheesbrough, 2004).

A total of 400 morning stool samples were taken from patients with gastrointestinal troubles attended the local health units in the same center referred to the laboratory by the clinician. A medical sheet was filled out on each one included age, sex, education, symptoms, water supply, and sewage disposal.

Stools were macroscopically examined for pin worms and gravid segments and microscopically by direct smears and by sedimentation and Kato-Katz techniques stained by MZN & trichrome (Othman, 2013). Quick immunochromatographic test was used for qualitative determination of *Cryptosporidium, Entamoeba,* & *Giardia* in stool samples (RIDA®QUICK ICA Combi, R Biopharm, Da-Rmstadt, Germany). This rapid diagnostic test (RDT) is a single-step, lateral-flow; immune-chromatographic assay (ICA) detected specific antigens of these protozoa parasites in a single test format viaAg binding to specific colored latex bound Abs. Patients on parasitic treatment or drugs in the last two weeks were excluded.

Statistical analysis: Data were collected and analyzed using SPSS software version 18.0. *P* value ≤ 0.05 was significant. Categories difference was compared using Pearson's Chi-square test (and Fisher's exact test

Results

The results were shown in tables (1 to 7).

Table 1: Parasites contaminated vegetable samples (n=60).

Vegetables	No. positive	One	Two	Three
Lettuce	31 (51.6%)	28 (46.7%)	2 (3.3%)	1 (1.7%)
Arugula	28 (46.6%)	24 (40.0%)	4 (6.6%)	0
Green onion	15 (25%)	15 (25%)	0	0
Leek	33 (55%)	31 (51.7%)	2 (3.3%)	0
Parsley	36 (60%)	33 (55%)	2 (3.3%)	1 (1.7%)
Radish	24 (40%)	24 (40%)	0	0
Total	167 (46.38%)	155 (43.1%)	10 (20%)	2 (3.3%)

Chi-square statistic =17.7186. P-value = 0.001401

Table 2: Prevalence of infection according to vegetable source

Villages	No. examined	No. positive (%)
Sinro, Ibshway Center.	100	41 (41%)
Sinhour, Snores Center.	100	54 (54%)
El-Khamseen, Etsa Center	100	62 (62%)
Fayoum City markets	60	10 (16.7%)
Total	360	167 (46.38 %)

Chi-square = 34.6094. P-value= < 0.00001

Table 3: Types of contaminated parasites on vegetables examined

Detected parasite	Lettuce	Arugula	Green onion	Leek	Parsley	Radish	Total (%)
Fasciola spp. EMC	1	2	0	4	3	1	11 (3.1%)
Ascaris lumbericoides eggs	5	4	3	3	7	4	26 (7.2%)
Toxocara canis	4	3	0	3	2	4	16 (4.4%)
Trichuris eggs	0	0	0	2	1	0	3 (0.83%)
Cryptosporidiumpaqrvum	9	6	0	7	7	3	32 (8.9%)
Entamoeba histolytica/dispar	4	6	5	5	4	4	28 (7.8%)
Entamoeba coli	8	9	7	8	8	4	44 (12.2%)
Giardia lamblia	3	2	0	2	5	2	14 (3.9%)
Cyclospra species	1	0	0	1	1	1	4 (1.11%)
Cystoisospora belli	0	0	0	0	2	1	3 (0.83%)
Total	35	32	15	35	40	24	181(50.3%)

Table 4: Types of contaminated parasites relation to Centers of villages

Detected parasite	Ibshway	Snores	Etsa	Fayoum	Total	Chi-square	p-value
Fasciola spp. EMC	2	4	4	1	11 (3.1%)	3.2727	0.351
A.lumbericoides	5	8	12	1	26 (7.2%)	13.3333	0.003*
T. canis	3	3	8	2	16 (4.4%)	7.3333	0.061
Trichuris eggs	0	1	2	0	3 (0.83%)	1.6593	0.646
C. parvum	8	12	10	2	32 (8.9%)	9.3333	0.025*
E.histolytica/dispar	7	7	12	2	28 (7.8%)	9.5238	0.023*
E. coli	14	16	12	2	44 (12.2%)	14.0606	0.002*
G. lamblia	3	4	4	3	14 (3.9%)	0.381	0.944
Cyclospra species	1	1	2	0	4 (1.11%)	0.80	0.849
Cysto. belli	1	1	1	0	3 (0.83%)	0.11	1.00

*significant difference

Table 5: Demographic date of patients according to centers.

Items	Characters	Ibshway	Snores	Etsa	Fayoum City	Total (%)	p-value
Age	<16years	59	64	28	55	206 (51.5)	0.457
	≥16years	41	36	72	45	194 (48.5)	
Sex	Male	66	64	51	49	210 (52.5)	0.175
	Female	34	36	49	51	190 (42.5)	
Education	Literate	35	36	23	67	161 (40.2)	0.041*
	Illiterate	65	64	77	33	239 (54.8)	
Pure water supply	Absent	65	57	60	35	217 (54.3)	0.032*
	present	35	43	40	65	183 (45.7)	
Sewage disposal	Sanitary	30	33	23	80	166 (41.5)	0.035*
	Unsanitary	70	67	77	20	234 (58.5)	
Total		100	100	100	100	400	

Table 6: Parasitic Infection distribution among patients according to centers

Parasites	Ibshway	Snores	Etsa	Fayoum	Total (%)	Chi-sq	p-value
Fasciola spp.	4	6	5	2	17(4.3%)	2.0809	0.0255*
A. lumbericoides	10	9	14	2	35 (8.8%)	6.5435	0.0435*
Trichuris eggs	1	2	3	2	8 (2.0%)	0.768	0.800
C. parvum	12	17	12	7	48 (12.0%)	4.2955	0.013*
E. histolytica/dispar	14	10	13	7	44 (11.0%)	2.8044	0.014*
E. coli	21	21	17	10	69 (17.4%)	4.453	0.034*
G. lamblia	6	7	10	3	24 (6.0%)	5.336	0.023*
Cyclospra species	0	0	2	0	2 (0.5%)	8.056	0.778
Cystois. belli	0	0	2	0	2 (0.5%)	8.056	0.778
Total and percent	68 (68%)	72 (72%)	78 (78%)	31 (31%)	249 (62.3%)	7.873	0.012*

*significant difference

Table 7: Sensitivity and specificity of ICA techniques

Parasites	Total (%)	Positive cases by each method (n)			Sensitivity	Specificity
Fasciola spp. EMC	17(4.3)	Microscopy (17)				
A. lumbericoides	35 (8.8)	Microscopy (35)				
Trichuris eggs	8 (2.0)	Microscopy (8)				
*C. parvum**	48 (12.0)	Microscopy (10)	MZN (40)	ICA (48)	100**%**	95.0**%**
E. histolytica/dispar *	44 (11.0)	Microscopy (12)	Trichrome (33)	ICA (44)	100%	96.5%
G. lamblia *	24 (6.0)	Microscopy (13)	Trichrome (19)	ICA (24)	100%	98.0%
E. coli	69 (17.4)	Microscopy (22)	Trichrome stain (69)			
Cyclospra species	2 (0.5)	Microscopy (1)	MZN stain (2)			
Cystoisospora belli	2 (0.5)	Microscopy (1)	MZN stain (2)			
Total	249 (62.3)					

*ICA sensitivity and specificity compared to staining as a gold standard

Discussion

No doubt, irrigation with contaminated water became a painful reality in some villages in Fayoum Governorate due to lack of clean canal water and contamination by improper sewage disposal.

In the present study, parsley showed the highest parasitic contamination (60 %), followed by leek (55%), lettuce (51.6), Arugula (32%), Radish (24%) & Green onion (25%). This variance in parasitic contamination may be related to the exposure and agricultural conditions as the amount and frequency of irrigation and the distance between the edible part and the water surface.

In the present study, there was significant increase in parasitic contamination in villages of El-Khamseen, Sinhour and Sinro (62, 54, & 41% respectively), which belong the largest centers in Fayoum governorates which are notorious of water scarcity and unsanitary sewage disposal, samples from central Fayoum local markets showed the lowest prevalence (16.7%) as there markets receive vegetable from many different fields around the center with the relative availability of water and proper sewage disposal.

In the present study, eleven parasites were detected in samples, the incidence of parasites found in overall samples reaches 50.3%, which was higher than other studies in Egypt; 29.3% (Hassan *et al*, 2012), 31.7% (El-Said, 2012) and 16.1% (Ahmad *et al*, 2014), but agreed with incidences in Iran (Shahnazi *and* Jafari-Sabet, 2010**),** Phillipines (Sia *et al*, 2012), Nigeria **(**Omowaye and Audu, 2012) and southwest Ethiopia (Tamirat *et al,* 2014**)**. The incongruity might be attributed to geographical locations, climatic and environmental conditions, or differences sample size, techniques used, and post-harvest handling methods.

In the present study*, th*e non-pathogenic *E. coli* was the most prevalent 12.2% followed by *C. parvum* 8.9%, *E. histolytica/dispar* in 7.8% and the least was *Giardia* 3.9%. Dalle *et al.* (2003) in France considered parasites as markers for sewage contamination with animal and human excreta which cysts were very resistant and survived in water or soil under favorable conditions for months or years. Alum *et al*. (2014) in USA reported that the parasitic rates could be used in models for public health decision-making process and highlight the mitigation role of hand hygiene agents in their prevention and control. *Cyclospora* and *Cystisospora* gave lowest prevalence (1.11% & 0.83%) respectively due to their small size and difficulty to detect on light contamination (Garcia, 2007). In Egypt, *I. belli* was reported (Rifaat and Salem, 1963), and (0.47%) polluted surface water in Dakahlia (El Shazly et al, 2007) and *Cystoi. belli* (1.2%) among municipal waste collectors in Alexandria (Eassa *et al,* 2016)

In the present study, *A. lumbricoides* was 7.2%. It is one of the worldwide helminth with about 807-1,121 million infection (CDC, 2020). *Ascaris* nature eggs remained viable in soil for many years (Asaolu and Ofoezie, 2018). The second most prevalent contaminant was *T. canis* 4.4%, a soil-transmitted zoonotic parasite by ingestion of *T. canis* larvated eggs (Abou-El-Naga, 2018). *T. canis* among man and pet dogs as well as in canal water and soil were reported by many Egyptian authors (Rifaat *et al,* 1969; Haridy *et al,* 2009; Etewa *et al,* 2016; Morsy, 2020). The third most frequent helminthic parasite was *Fasciola* spp. 3.1%. Abou-Madyan *et al.* (2003) in Etsa Center reported 1.7% of 1019 individuals with fasc-ioliasis with geometric mean egg count of 33.2/gm. stools, and added that about 23.5% of tpatients were asymptomatic. A first *Fa-sciola* focus was in Abis village, Alexandria (Farag *et al,* 1979). Fascioliasis increased in many governorates (Haridy *et al,* 1999). *Trichuris trichuria* was 0.83%, of 604-795 million cases worldwide (CDC, 2020)

In the present study, the 400 patients in the corresponding local health units didn't show significant differences as to age and sex but significant association with literacy, availability of pure water supply and proper sewage disposal with increased prevalence of illiteracy, absence of piped water and insanitary sewage disposal in villages compared with Fayoum center (P <0.05)

In the present study, 62.3% of them had parasitic infection with significant increase in El-Khamseen village (78%) followed by Sinhour and Ibshaway compared to Fayoum center (31%). As to protozoa *E. coli* was the most prevalent 69 (17.4%) followed by *C. parvum* 48 (12%), *E. histolytica/dispar*44 (11.0%), *G. lamblia* 24 (6.0%) and lastly *Cyclospra* species and Cystoisopora belli 2 each (0.05%). As to helminthes, *A. lumbricoides* was the most prevalent 35(8.8%), followed by *Fasciola* spp. 17(4.3%) and then *Trichuris* 8 (2.0%).

In the present study, microscopy easily detected all helminthic eggs, but for *C. parvum* MZN stain was superior to microscopy, Trichrome stain was significant better than microscopy for *E. histolytica/dispar and G. lamblia,* but without significant with *E. coli*

In the present study, *C. parvum, E. histoly tica/dispar* and *Giardia* ICA rapid test compared to stain as a gold standard, gave 100% sensitivity & 95-98% specificity. Amarogentin, a biologically active secoiridoid glycoside for gentianaceae efficacy based medications with significance for batch to batch quality controls (Nuntawong *et al,* 2021).

The infectivity of contaminated vegetables or water pollution was studies in Dakalia (El Shazly *et al,* 2007), Alexandria (El-Said, 2012), Kalyobia (Ahmad *et al*, 2014), Middle Nile Delta (El-Kowrany *et al,* 2015) and Sharkia (Etewa *et al,* 2016), and abroad, in Libya (Abougrain *et al*, 2010), Saudi Arabia (Al-Megrin and Wafa, 2010), and Iraq (Hadi, 2011), with different contamination.

Conclusion

Sewage water in irrigation led to increased vegetables contamination with zoonotic parasites. Proper health education is a must. ICA is simple practical single step to detect parasites. While entering the era of water scarcity and continuing increase in population we have to figure out new and cheap methods for treating sewage water used for irrigation, law legislation and restricting this type of water for certain types of plants.

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