

Prevalence of Pathogenic Intestinal Parasites in Common Raw Edible Vegetables in Gezira State, Sudan: A Cross-sectional study

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Abstract: **Background:** Fresh vegetables are eaten raw and may also be a source of food-borne parasitic infections. This study aimed to detect the prevalence of intestinal parasites in raw edible vegetables in Wad Medani central vegetables market. **Methods:** A total of 90 samples of seven raw edible vegetables were selected and examined using formal ethyl acetate concentration technique, zinc sulphate flotation technique and modified cold Ziel-Neelsen stain, these vegetables were garden rocket, snake cucumber, carrot, lettuce, green onion, green pepper and tomato. **Result:** the overall contamination was 27.7% and contamination rate for each vegetable 45%, 33.3%, 30%, 40%, 27.3%, 8.3% and 0% for garden rocket, snake cucumber, carrot, lettuce, green onion, green pepper and tomato respectively, vegetables were categorized to leafy, peeled and others, the rate of contamination for each category was 52%, 44% and 4% respectively. Also some vegetables samples were taken before and some samples after distribution in the market by the local sellers, vegetables before distribution show 16.16% contamination and after distribution show 36.36% contamination. The parasites detected were *Giardia lamblia* cyst, *Entamoeba histolytica* cyst, *Entamoeba coli* cyst, *Taenia* species egg and *Hymenolepis nana* egg, the prevalence of these parasites were 48%, 16%, 12%, 12% and 12% respectively. 11(44%) of the parasites were detected by formal ethyl acetate concentration technique and 14 (56%) by zinc sulphate flotation concentration technique. There is no oocyst of *Cryptosporidium parvum* or *Isospora belli* detected using modified Ziel-Neelsen stain. **Conclusion:** This study concluded that there is high contamination of vegetables by intestinal parasites suggesting a great risk of acquiring intestinal parasitic infection by eating improperly washed vegetables.

Keywords: Edible Vegetables, Intestinal parasites, Sudan

Introduction

Pathogenic intestinal parasites are an organism that depends on a host to get its food from or at the expense of a host. Intestinal parasitic infections are widely distributed throughout the world, causing substantial danger to the public health, economical, physical and cognitive development particularly among children in developing countries like Sudan. The poor personal hygiene, and poor environmental and health system commonly observed in developing countries make the prevalence to be the highest among these populations (Tomass & Kidane, 2012), (Wegayehu, Tsalla, Seifu, & Teklu, 2013) et al., (Alade, Alade, & Adewuyi, 2013), (Punsawad, Phasuk, Thongtup, Nagavirochana, & Viriyavejakul, 2019)

Fresh vegetables are important part of healthy diet; they provide essential vital elements like vitamins, minerals and fibers. In many countries such vegetables are eaten raw or slightly cooked, this practice may also be a source of food-borne parasitic infections (Erdog̃rul & Şener, 2005) (Olyaei & Hajivandi, 2013), (Benti & Gemechu, 2014). Food normally becomes a potential source of human infections by contamination during production, collection, transportation or during preparation and processing. The source of contamination is usually faeces, faecally contaminated soil or water (Slifko, Smith, & Rose, 2000); (Daryani, Ettehad, Sharif, Ghorbani, & Ziaei, 2008); (Damen, Banwat, Egah, & Allanana, 2007), (Alhabbal, 2015). With the increasing consumption of fresh vegetables there has been a corresponding rise in the number of food borne illnesses in some countries (Klapec & Borecka, 2012), (Maqbool, Khan, Yasmin, & Sultana, 2014).

Several surveys in different parts of the world indicated that the vegetables can be an agent for transmission of protozoan cysts and oocysts (*Giardia*, *Entamoeba*, *Cryptosporidium*, *Cyclospora*, *Toxoplasma* and *Isospora*) and helminthes ova's and larvae

(*Hymenolepis spp*, *Taenia spp*, *Fasciola spp*, *Toxocara*, *Ascaris*, *strongyloides* and *Hook worms*) (Anh, Tram, Klank, Cam, & Dalsgaard, 2007); (Darchenkova, Romanenko, & Chernyshenko, 2006); (Germano, 1992), (Nazemi, Raei, Amiri, & Chaman, 2012). Epidemiological studies have also indicated that, in areas of the world where parasitic diseases are endemic in the population and where wastewater is used to irrigate vegetables which are eaten raw, the consumption of wastewater irrigated vegetables without proper washing may lead to parasitic infection (Damen et al., 2007). In Sudan the raw edible vegetables are highly consumed, especially in an irrigate scheme in Gezira state, where many types of vegetables are planted.

Methods

Study design

The study was cross-sectional, aimed to determine the prevalence of pathogenic intestinal parasites in the selected raw edible vegetables.

Study area

The study was conducted in Wad Medani central vegetables market, Wad Medani, Gezira state, Sudan. Wad Medani city which located in Central Sudan in the western bank of the Blue Nile River, in 187 Km south of Khartoum. Wad Medani is the main city of the irrigated area of Gezira Agricultural Scheme, where different types of vegetables are cultivated and distributed to other states in Sudan mainly Khartoum state. Some farmers in Gezira Agricultural Scheme use natural fertilizers that may be a source of infection with pathogenic parasites

Sample Size:

Ninety samples from seven selected raw edible vegetables were collected randomly, all selected samples were taken before and after distribution by the local sellers in the market; Garden rocket (20 samples), Green onion (11 samples), Green pepper (12 samples), Tomato (12 samples), Carrot (10 samples), Lettuce (10 samples) and Snake cucumber (15 samples).

Sample processing:

About 200 grams of each sample were taken and soaked in about 100 ml physiological saline for half an hour, the suspension was strained through sieve in to a clean beaker to remove un-desirable materials. The filtrate was divided into two vials one for formal ethyl acetate centrifugation sedimentation technique and the other for zinc sulphate flotation technique.

Formal Ethyl acetate Centrifugation Sedimentation Method:

The filtrate obtained from the sample processing step was centrifuged at 5000 rpm for 5 minutes, the supernatant was discarded, and then 4 ml of 10% formal water was added to the sediment and mixed well. Three ml of ethyl acetate were added to the mixture, the suspension was centrifuged at 5000 rpm for 1 minute and the supernatant was discarded. wet mount was prepared from the sediment on a clean microscopic glass slide with a drop of lugoul's iodine and carefully covered with a cover slip, then examined under the light microscope using 10X and 40X objective lenses.

Zinc Sulphate Flotation Concentration Method:

Three to four ml 33% zinc sulphate solution (specific gravity 1.18) was added to the sediment (as prepared above) and mixed well in a conical tube and more zinc sulphate solution was added to fill the conical tube within 5mm of the top and was centrifuged again for 2 minutes. The tube was carefully removed from the centrifuge and placed vertically in a rack. After 1-2 min, a wire loop, of approximately 5 mm in diameter, was touched to the surface film removing several loops full and transferred to a glass slide. A drop of lugoul's iodine was added and covered with cover glass. The slide was examined under light microscope using 10X and 40X objective lenses and the results were recorded.

Modified Cold Ziehl-Neelsen Stain Method

A drop from the above sediment was obtained and placed in a clean slide and a smear was made. Then the smear was air dried and fixed with methanol for 2 minutes. The modified ZN was done according to (Cheesebrough, 1991), the film was examined under microscope using 100x oil immersion objective lens and the results were recorded.

Statistical analysis:

Data were analyzed using Statistical Package for Social Sciences (SPSS) software program version (20.0).

Results:

Out of 90 samples 25 (27.7%) were found positive by microscopic examination for intestinal parasites using formal ethyl acetate and zinc sulphate concentration techniques. *G.lamblia* cyst recovered in 12 samples representing (48%), *E.histolytica* cyst 4 positive samples representing (16%), *E.coli* cyst 3 positive samples representing (12%), *H. nana* egg 3 positive samples representing (12%) and *Taenia* species egg 3 positive samples representing (12%) (Table 1). No oocysts of *C.parvum* or *I. belli* were recovered using ZN stain. The protozoan parasites were detected 19 out of 25 (76%) positive samples, while intestinal helminthes detected in 6 out of 25(24%) of the entire positive samples. ZN stain was used to differentiate between *taenia* egg species, and revealed *Taenia saginata*. The highest contamination in vegetables was found in garden rocket 9/20 samples (45%), followed by lettuce 4/10 samples (40%), snake cucumber 5/15 samples (33.3%), carrot 3/10 samples (30%), green onion 3/11 samples (27.3%), green pepper 1/12 sample (8.3%) and there is no parasite recovered in the 12 tomato samples (Table 2). Forty four Out of 90 samples (48.9%) were collected before backing and distribution in the market by the local sellers and 46/90 samples (51.1%) were collected after distribution. Out of the 44 samples that were collected before distribution 8/44 (18.18%) were found positive and out of 46 samples that were collected after distribution 16/46 (36.6%) were found positive (Tables 3).Vegetables were categorized to leafy; Garden rocket and lettuce, pealed vegetables; snake cucumber, green onion and carrot and other vegetables; tomato and green pepper, the highest contamination was in leafy vegetables (52%) followed by pealed vegetables (44%) and the least contamination was in other vegetables (4%) (Table 5). From all 25 positive samples, the parasites that recovered by sedimentation method were 11/25(44%) parasite and the parasites recovered by floatation method were 14/25 (56%) parasite (Table 6).

Table (1): Frequency of intestinal parasites detected in selected vegetables

Parasite	Frequency	Percent %
<i>Giardia lamblia</i> cyst	12	48
<i>Entamoeba histolytica</i> cyst	4	16
<i>Entamoeba coli</i> cyst	3	12
Taenia egg	3	12
<i>Hymenolepis nana</i> egg	3	12
Total	25	100

Table 2: Distribution of intestinal parasites among the examined vegetables

Vegetables	No Examined	Parasites detected					No(%) Of parasite detected
		<i>Giardia lamblia</i> cyst	<i>Entamoeba histolytica</i> cyst	<i>Entamoeba coli</i> cyst	<i>Hymenolepis nana</i> egg	Taenia saginata	
Snake cucumber	15	3	1	1	*ND	*ND	5(33.3%)
Garden rocket	20	5	1	1	1	1	9(45%)
Tomato	12	*ND	*ND	*ND	*ND	*ND	0(0%)
Carrot	10	1	1	*ND	*ND	1	3(30%)
Green pepper	12	1	*ND	*ND	*ND	*ND	1(8.3%)
Lettuce	10	1	1	*ND	1	1	4(40%)
Green onion	11	1	*ND	1	1	*ND	3(27.3%)
Total	90	12	4	3	3	3	25(27.7%)

*ND: Not detected

Table 3: Prevalence of intestinal parasites among vegetables before distribution

Vegetables	No Examined	Parasites detected					No(%) of parasite detected
		<i>Giardia lamblia</i> cyst	<i>Entamoeba histolytica</i> Cyst	<i>Entamoeba coli</i> cyst	<i>Taenia saginata</i>	<i>Hymenolepis nana</i> egg	
Snake cucumber	7/15	*ND	*ND	1	1	*ND	2(28.57%)
Garden rocket	10/20	1	*ND	*ND	*ND	1	2(20%)
Tomato	6/12	*ND	*ND	*ND	*ND	*ND	0(0%)
Carrot	5/10	*ND	*ND	*ND	*ND	1	1(20%)
Green pepper	6/12	*ND	*ND	*ND	*ND	*ND	0(0%)
Lettuce	5/10	*ND	*ND	*ND	*ND	1	2(40%)
Green onion	5/11	*ND	*ND	*ND	*ND	*ND	1(20%)
Total	44/90	1	*ND	1	3	3	8(18.18%)

*ND: Not detected

Table 4: Prevalence of intestinal parasites among vegetables after distribution in the market by the local sellers

Vegetables	No Examined	Parasites detected					No(%) of parasite detected
		<i>Giardia lamblia</i> cyst	<i>Entamoeba histolytica</i> Cyst	<i>Entamoeba coli</i> cyst	<i>Taenia saginata</i>	<i>Hymenolepis nana</i> egg	
Snake cucumber	8/15	3	1	*ND	*ND	*ND	4(50%)
Garden rocket	10/20	4	1	1	*ND	*ND	6(60%)
Tomato	6/12	*ND	*ND	*ND	*ND	*ND	0(0%)
Carrot	5/10	1	*ND	*ND	*ND	*ND	1(20%)
Green pepper	6/12	1	*ND	*ND	*ND	*ND	1(16.6%)
Lettuce	5/10	1	1	*ND	*ND	*ND	1(20%)
Green onion	6/11	1	*ND	1	*ND	*ND	2(33.3%)
Total	46/90	11	3	2	*ND	*ND	16(36.36%)

*ND: Not detected

Table 5: Frequency of intestinal parasites among leafy vegetables, peeled vegetables and other vegetables

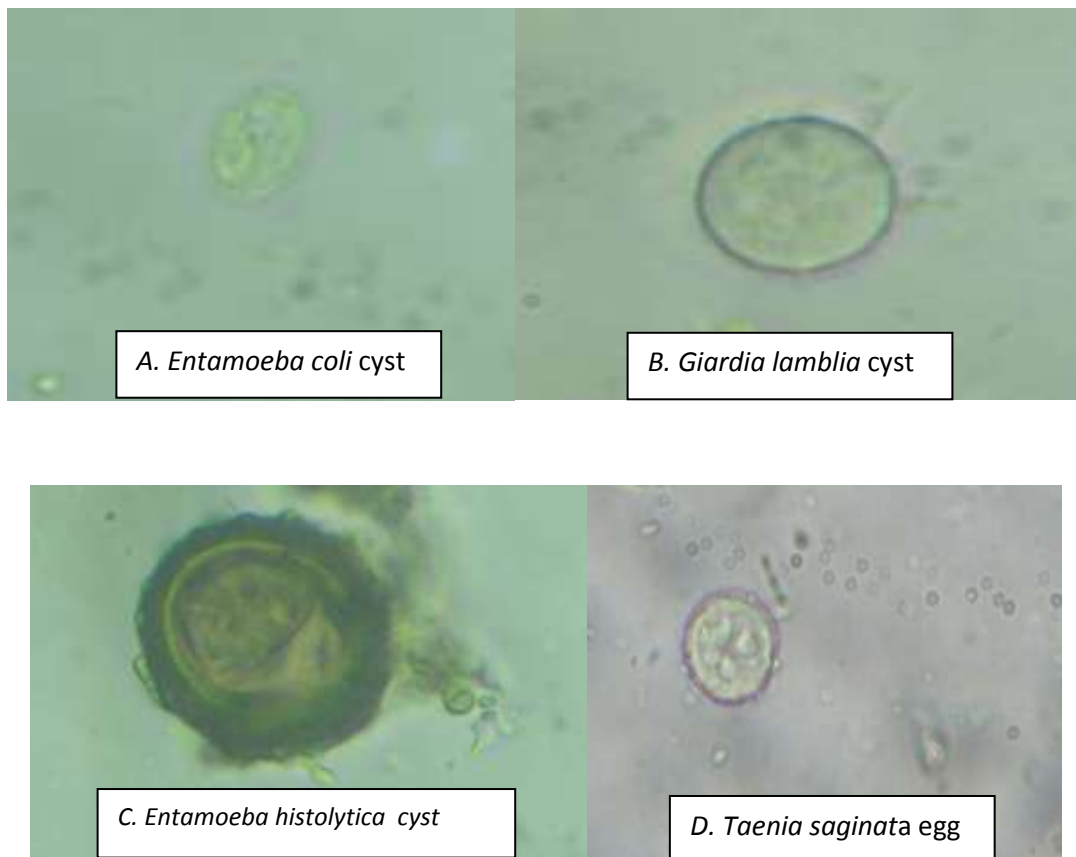
Type of vegetable	Frequency of intestinal parasites detected	Percent %
Leafy	13	52%
Peeled	11	44%

Others	1	4%
Total	25	100%

Table 6: Comparison between sedimentation and flotation concentration techniques in detection of parasites

Technique used	No of parasites detected	Percent %
Sedimentation	11	44%
Flotation	14	56%
Total	25	100%

Microphotography: *Giardia lamblia* cyst in garden rocket sample *Entamoeba coli* cyst in green onion sample , *Entamoeba histolytica* cyst in snake cucumber sample and *Taenia saginata* egg in lettuce sample



Discussion:

Several studies were conducted to detect the contamination of vegetables with intestinal parasites, in the current study, the overall contamination rate was found to be 27.7%, which is higher than what was reported in Saudi Arabia, 16.4% (Al-Megrm, 2010), Egypt 19.4% (El Said Said, 2012) and Sudan 13.5% (Mohamed, Siddig, Elaagip, Edris, & Nasr, 2016). The overall contamination rate was found to be lower when compared with the findings of some studies reported in Egypt 31.7%;(Hassan, Farouk, & Abdul-Ghani, 2012), 32.6% in Iran (Daryani et al., 2008), 58% in Libya (Abougrain, Nahaisi, Madi, Saied, & Ghenghesh, 2010), and 75.9% in Kenya (Nyarango, Aloo, Kabiru, & Nyanchongi, 2008).

In the present study *Giardia lamblia* cyst is the most protozoan parasites recovered, comprised about 48% of the total parasites detected; which was considered to be higher than what was detected in Egypt (6.7%), and Saudi Arabia (31.6%) (El Said Said, 2012), (Al-Megrm, 2010). This could be due to the majority of workers whom backing the vegetables are children; they cheaper

than the adult workers, many studies revealed that *Giardia lamblia* are more prevalent among children in Sudan (Muhajir, Hajissa, Mohamed, & Aal, 2017). *E. histolytica* cysts represent about 16% of the total parasites detected, which was considered to be higher than that detected in Iran (8%), Nigeria (14%), and lower than that detected in Riyadh 35.5%, (Damen et al., 2007) (Al-Megrm, 2010), 2010, (Al-Shawa & Mwafy, 2007). The percentage of *E. coli* was 12%, which was higher than what was detected in Manila, Philippines 2.5% (Su et al., 2016).

Taenia saginata and *H. nana* eggs were recovered in the current study 12% for each one, *H. nana* eggs been present was in consistent with what was found in Libya (Abougrain et al., 2010), In other studies these two eggs were recovered with other helminthes eggs and larvae (Darchenkova et al., 2006); (Germano, 1992), (Kozan, Gonenc, Sarimehmetoglu, & Aycicek, 2005) and disagreed with the result reported zero percent prevalence of helminthic eggs in Turkey.

Our study was conducted in the rainy season, and many studies showed that different seasons play an important role in the presence of eggs and larvae among vegetables. The egg and larvae may washed away; by rain from the surface of vegetables (Uga et al., 2009). Also another reason for these differences may be due to the difference in hygiene of workers, sample size, the ways of irrigation and distribution of parasite worldwide. *C. parvum* and *I. belli* oocysts were not recovered in the current study. This was disagree with (Razavi, Nasirinasab-Rafsanjani, & Bahrami, 2010) in a study done in Iran report that oocyst of *Cryptosporidium* were recovered in 23.5% of collected lettuce and also, in Poland, *Cryptosporidium* oocysts were detected in 4.69% of vegetable samples (Rzeżutka et al., 2010). These differences may be due to the limitation of method used to identify the oocyst and/or the distribution of parasite worldwide.

The high contamination of leafy vegetables might be due to their broad leaves that provide large contact area with contaminated irrigation water and soil (Larkin et al., 1978), leafy vegetables had uneven surfaces that make parasitic eggs, cysts and larvae attached to their surface more easily when washed with contaminated water either in the farm or market (Avcioglu, Soykan, & Tarakci, 2011); (Amoah, Drechsel, Abaidoo, & Ntow, 2006); (Kozan et al., 2005).

The least contamination of parasites was found in other vegetables; tomato and green pepper 8.3%, these observations are in accordance with (Uga et al., 2009). The low contamination may be due to the fact these vegetables have smooth surface, which make the removal of parasites cysts, larvae and eggs so easy when washed and also may be due to the uncontrolled use of agricultural pesticide for tomato.

In the current study some of vegetable samples were collected before distribution by the local sellers with 18.18% contamination and some samples after distribution, which show higher contamination 36.36%, this might be due to the attitude of the market sellers that they spraying the vegetables with water, which might be contaminated with intestinal parasites and/or poor hygiene of market sellers.

Comparing the two methods used in the present study (sedimentation and flotation), it was observed that flotation method had the highest recovery rate 56% while sedimentation is 44% , which is not in a line with (Damen et al., 2007) who had a high recovery rate by sedimentation. This might be due to the types of parasites recovered, types of vegetables examined, the way of transporting and handling of vegetables and environmental conditions.

Conclusion

The results from this study showed high contamination of vegetables with intestinal parasites suggesting existence of a great risk of acquiring intestinal parasitic infections especially protozoan parasites by eating improperly washed vegetables.

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