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Short Communication

Parasitic Contamination of Fresh Vegetables Sold at Upper and Lower Dir Districts, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Current study was aimed to determine the prevalence of parasitic infection in vegetables collected from the vegetable markets in Lower and Upper Dir districts. A total of 520 vegetable samples were collected during April to October 2016. These vegetable samples were screened for parasitic contamination. The results showed that 10.7% of the vegetables were found to be contaminated with one or more than one species of parasites. Intestinal parasites *Ascaris lumbricoides* 26.7%, *Trichuris trichura* 19.6%, *Taenia saginata* 25 % and *Entamoeba histolytica* cyst 28.5% were detected. The highest prevalence was recorded for *Entamoeba histolytica* while *Trichuris trichura* was detected as the least parasitic infection. *Coriandrum annum* (Coriander) was found highly contaminated 14.2% with parasitic infection while *Zingiber officinale* (Ginger) with least 1.78%. The vegetable collected from lower Dir were highly infected 41(73.2) than the upper Dir district 15(26.7). Based on the results of the present study it was suggested that vegetables are the possible source of transmission of intestinal parasites.

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Authors' Contributions
WK conceived and designed the study. GM, SB and SA performed the study. WK, GM, SB and SA analyzed the data. WK wrote the manuscript.

Key words
Raw vegetables, Parasitic contamination, Unhygienic, Poor sanitation, Geohelminth.

Due to the nutritional value vegetable is the essential component of a healthy human diet. Consumption of raw vegetables and salads is a common practice, as they retain natural flavor and preserve heat labile nutrients. On the other hand, vegetables can act as a potential source for several parasitic and infectious diseases (Izadi *et al*., 2006; Idahosa, 2012). It has been estimated that approximately 3.5 billion people are affected and that 450 million are sick from intestinal parasite infections, with an estimated 200,000 deaths annually in the world (Walked, 2009). Fresh veggies can be the factors of transmission of protozoan cysts and helminth eggs and larvae (Gharavi *et al*., 2002; Daryani *et al*., 2008).

Epidemics of intestinal parasitic infections are associated with raw vegetables have been reported from developing as well as developed countries of the world. In Africa, the transmission of intestinal parasitic infection has been considered to increase successfully due to the frequent use of untreated human or animal dung as manure in cultivation by the local farmers, which serves as a source of zoonotic parasitic infection (Luca *et al*., 2000). Eating raw vegetables is common in Pakistan. The present study describes the prevalence of parasitic contamination and their possible association with vegetables eaten raw in Lower and Upper Dir districts.

Materials and methods

A total of 520 fresh vegetable samples were collected randomly from two main vegetable markets one each in Lower (320 samples) and in Upper Dir (200 samples) districts. The vegetables are brought from different farms and agricultural fields of Malakand division, where majority of the farmers use human and animal manures to extend the commercially processed fertilizer to limit their cost of farming.

The vegetable samples collected for this study were *Solanum tuberosum* (potato), *Zingiber officinale* (ginger), *Allium cepa* (onion), *Brassica compastris* (mustard), *Abelmoschus esculanta* (lady finger), *Colocasia esculenta* (taro), *Coriandrum annum (*coriander*)*, *Allium sativum* (garlic), *Spinacia oleracea* (spinach), *Lycopersicum esculentum* (tomato), *Capsicum annum* (chilli), *Daucus carota* (carrot) and *Cucumis sativus* (cucumber). Each sample were washed in formalin for the removal of the parasite ova, larva or cysts. The suspension was strained through a cheese cloth to remove undesirable materials. A drop of the pellet was placed on a clean slide and examined under the microscope using 10X and 40X objectives. For the confirmation of the parasite the sedimentation and floatation methods were applied (Gharavi *et al*., 2002).

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Table I.- Prevalence rate of intestinal parasitic contamination in different fresh vegetables among the two major markets in Lower and Upper Dir districts (April to October 2016).

Type of vegetable	Vegetable market in Lower Dir		Vegetable market in Upper Dir		Total examined	Total contaminated (Infection %)
	No.of examined	No. of positive (%)	No. of examined	No.of positive (%)		
Solanum tuberosum (Potato)	29	3	19	1	48	4 (7.14)
Allium sativum (Garlic)	26	4	18	2	44	6 (10.7)
Zingiber officinale (Ginger)	22	1	19	0	41	1(1.78)
Allium cepa (Onion)	18	2	15	1	33	3(5.35)
Daucuscarota (Carrot)	31	3	12	1	43	4(7.14)
Colocaciaesculenta (Taro)	23	5	8	2	31	7(12.5)
Brassica compestriss (Mustard)	27	3	17	1	44	4(7.14)
Coriandrum annum (Coriander)	25	6	13	2	38	8(14.2)
Spinacia oleracea (Spinach)	24	5	14	0	38	5(8.92)
Lycopersicum esculentum (Tomato)	30	3	17	2	47	5(8.92)
Capsicum annum (Chili)	23	1	15	1	38	2(3.57)
Cucumis sativus (Cucumber)	16	2	16	1	32	3(5.35)
Abelmoschus esculantum (Lady finger)	26	3	17	1	43	4(7.14)
Total	320	41(73.2)	200	15(26.7)	520	56(100)

About 200g of each vegetable were washed with distilled water in a plastic container. The filtrate was decanted into a beaker while the remains were sediment in the bottom of the container. A drop of the sediments was examined under the microscope to investigate parasite stages. Similarly, the suspension was shaking with a glass rod for 5 min and added Sodium chloride and zinc sulfate regularly. After the regular shaking the supernatant layer was formed, a slide was taken on the upper surface of the tube and left it for 5 min, the slide was observed under the microscope for parasite evidence. The floatation fluid was examined under microscope using 10X and 40X objectives. The eggs/cysts were identified based on morphological details with the help of keys provided by Soulsby (1982).

Data analysis was performed using the Graph Pad Prism 5. One way analysis of variance ANOVA was used to find out the association between the acquisition of parasitic infection in fresh vegetables and type of parasites and to check the significance between the type of contaminated parasite and type of vegetable. The p value <0.005 was considered as significant.

Results and discussion

Protozoan cysts and helminth eggs were detected in 10.7% (n=56/520) of fresh vegetables examined (Table I). Interestingly, the most detected parasites in the vegetable samples in both markets were *E. histolytica / dispar* (28.5 %), *A. lumbricoides* (26.7 %), *T. saginata* (25%) and *T. trichura* (19.6 %) (Table II). The most contaminated vegetable was coriander (14.2 %) followed by taro (12.5%) and least number of parasites was detected in cucumber (1.78 %) (Table I). Furthermore, the rate of contamination in fresh vegetables examined in vegetable market, Lower Dir district was much higher (73.3 %) compared to that of Upper Dir district (26.7%) (Table I). No significant difference was found amongst the vegetables and that of the parasitic infection (Table II).

The daily consumed vegetables have been reported to be frequently contaminated with different protozoan and helminth parasites throughout the world (Said, 2012; Gupta *et al*., 2009). By comparing this result with other studies, it was lower than many studies done in Syria (Alhabbal, 2015), Iran (Nazemi *et al*., 2012; Ebrahimzadeh *et al*., 2013), Pakistan (Ul-Haq *et al*., 2014), Ethiopia (Tomass and Kidane, 2012; Tefera *et al*., 2014; Benti and Gemechu, 2014), Egypt (Said, 2012; Eraky *et al*., 2014), Nigeria (Idahosa, 2011; Alade *et al*., 2013; Simon-Oke *et al*., 2014), Vietnam (Uga *et al*., 2009) and Nigeria (Shehu and Amina, 2014). The variation in degree of contaminations may be attributed to geographical location, type and number of samples examined, methods used for detection, different laboratory techniques used, type of water used for irrigation, post harvesting handling methods of such vegetables and even the type of water used to wash vegetables can play an instrumental role in the epidemiology of transmission of parasitic diseases.

Eggs of 4 different species of parasites namely *A. lumbricoides*, *E. histolytica*, *T.saginata, T. trichura,* were detected in the vegetables examined during this study. In present study the most detected parasite was *E. histolytica/*

Table II.- Distribution of intestinal parasites in relation to the type of fresh vegetable samples collected from both the markets (April to October 2016).

Vegetables	*Taenia saginata*	*Ascaris lumbricoides*	*Entamoeba histolytica*	*Trichuris trichuira*	Total	P Value
Leafy (n=120)						
Brassica compestriss (Mustard)	2	1	0	1	4(7.14)	0.2437
Coriandrum annum (Coriander)	1	4	1	2	8(14.2)	
Spinacia oleracea (Spinach)	1	2	1	1	5(8.92)	
Sub-total	4	7	2	4	17(30.3)	
Mean ± SD	1.33± 0.57	2.33±1.52	0.66±0.57	1.33±0.57	5.66±2.08	
Root (n=240)						
Solanum tuberosum (Potato)	0	1	3	0	4(7.14)	0.2970
Zingiber officinale (Ginger)	1	0	0	0	1(1.78)	
Allium sativum (Garlic)	1	2	2	1	6(10.7)	
Colocaciaesculenta (Taro)	3	2	2	0	7(12.5)	
Daucuscarota (Carrot)	1	1	1	1	4(7.14)	
Allium cepa (Onion)	1	0	1	1	3(5.35)	
Sub-total	7	6	9	3	25(44.6)	
Mean ± SD	1.16±0.98	1±0.89	1.5±1.04	0.5±0.54	4.16±2.13	
Fruit (n=160)						
Lycopersicum esculentum (Tomato)	1	1	2	1	5(8.92)	0.3829
Cucumis sativus (Cucumber)	1	1	1	0	3(5.35)	
Capsicum annum (Chili)	0	0	1	1	2(3.57)	
Abelmoschus esculantum (Lady finger)	1	0	1	2	4(7.14)	
Sub-total	3	2	5	4	14(25)	
Mean ± SD	0.75±0.5	0.5±0.5	1.25±0.5	1±0.81	3.5±1.29	
Gross total	14(25)	15(26.7)	16(28.5)	11(19.6)	56	

dispar in fresh vegetable samples, and this result is in accordance with study conducted by Mohamed et al. (2016) and Benti and Gemechu (2014). *Zingiber officinale* (Ginger) and chili cucumber were found with least parasitic contamination, while Ul-Haq et al. (2014) found that cayenne pepper was less contaminated by parasitic contaminations in Lahore, Pakistan.

Ascaris lumbricoides was detected in 2.88% (n=15/520) of vegetables examined in the present work. A study from Saudi Arabia reported the detection of *A. lumbricoids* in 16% of leafy vegetables examined (Al-Binali *et al*., 2006). Another study from Iran, reported prevalence of *A. lumbricoids* eggs being detected in 2% of samples examined (Daryani *et al*., 2008). *Taenia saginata* 2.69 %(n=14/520) and *Trichuristrichura* 2.11% (n=11/520) were evidenced in this study.

In current study the coriander had the highest parasitic contamination which is not with agreement to the studies conducted by (Alhabbal, 2015; Sunil *et al*., 2014; Ul-Haq *et al*., 2014; Eraky *et al*., 2014) in that the lettuce had the highest parasitic contamination, this may be due to its leaves which are capable of sheltering parasites more easily than other vegetables *i.e. Zingiber officinale* (Ginger), chili, *Allium cepa* (Onion) and cucumber.

Unhygienic handling during transportation as well as use of contaminated water to wash the vegetable are the main sources of parasitic diseases. Fresh vegetable samples in this study were found to have one or more than one species of parasite which reflects the level of faecal contamination of vegetables which may result in several parasitic infections (Shehu and Amina, 2014). Besides that animal manure, faeces of wild jackals, foxes and stray dogs frequently visiting the fields, human defecation practices of rural population and post-harvest washing of the vegetables with dirty water also contribute to the contamination of vegetables. The high overall prevalence of parasitic contamination in vegetables indicates an alarmingly high risk for the consumers of the vegetables.

Conclusions

Fresh vegetables in Dir districts were moderately contaminated by intestinal parasites, which are source of infection with amoebiasis, ascariasis, taeniasis, trichuriasis and others. Effective and comprehensive prevention and control measures should be taken to ensure food safety.

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Statement of conflict of interest

Authors declare that they have no competing interests.

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