Pakistan J. Zool., vol. 46(5), pp. 1303-1309, 2014.

Parasitic Contamination of Vegetables Eaten Raw in Lahore

Shafa-ul-Haq,[1] Azhar Maqbool,[1] Umbreen Javed Khan,[2] Ghazalah Yasmin[3] and Razia Sultana[1]
[1]*Departments of Parasitology, University of Veterinary & Animal Sciences, Lahore*
[2]*Department of Zoology, Govt Post Graduate College for Women, Samanabad, Lahore*
[3]*Department of Botany,Govt College for Women, Jhelum*

Abstract.- Fresh vegetables are an important part of human diet. Eating raw vegetables is customary in many parts of the world including Pakistan, but these raw vegetables can be a major source of parasitic infection. A parasitic survey on vegetables collected from major markets and growing fields in Lahore was conducted for the discovery of human and animal parasites. Ten vegetables *viz.*, beet, cabbage, carrot, chili, coriander, cucumber, lettuce, mint, radish and tomato were evaluated in this study. Fifty sample of each vegetable were taken, comprising a total of 500 samples for the study. The collected samples were processed and microscopic examination was carried out for the presence of parasites on the vegetables. All vegetables were highly contaminated with parasites with an overall prevalence of 31.2%. Lettuce showed the highest contamination (48%), followed by cabbage (44%) and mint (podina) (42%) while chili showed the least contamination (16%). Examination of vegetables revealed twelve genera of parasites. Of parasites studied, *Ascaris* eggs found to be the highest (37.1%), followed by hookworm (10.8%) and *Trichostrongyloides* sp. (8.9%), and the least common parasite was *Toxoplasma gondii* (1.9%). The results would seem to indicates that one of the important routes of parasitic infection to humans is the consumption of raw and unwashed vegetables.

Key words: Raw vegetables, parasitic infection, prevalence of parasites.

INTRODUCTION

Fruits and vegetables are an important part of the diet providing vitamins, minerals, fiber proteins, essential micronutrients and other bio-functional components. With the increasing consumption of fresh fruits and vegetables there has been a corresponding rise in the number of food borne illnesses (Klapec and Borecka, 2012; Abourgrain *et al*., 2010). Vegetables can become contaminated with bacterial, viral and parasitic pathogens during harvest (due to the lack of sanitary facilities of workers or dirty storage capacity) or post harvest treatment (handling, storage and transportation) (Kirezieva *et al*., 2013; Amoah *et al*., 2007).

Organic fertilizers (sewage sludge, animal manure and compost) may also be a potential risk of contamination of fruits or vegetable (Bouhoum and Amahmid, 2002). Contamination may also occur when food, particularly salad vegetables and the fruit are rinsed in polluted potable water (Avicoglu *et al*., 2011). Fresh vegetables can be agents of transmission of protozoan cysts and helminth eggs and larvae (Daryani *et al*., 2008; Erdogrul and Sener, 2005; Coelho *et al*., 2001). Outbreaks of intestinal parasitic infections epidemiologically associated with the consumption of raw vegetables have been reported from developed and developing countries (Ortega *et al*., 1997; Mintz *et al*., 1993; Zahid *et al*., 2004).

Parasitic infection are often not routinely considered as a source of illness, especially when the symptoms are similar to a bacterial or viral infection. Therefore, in many instances parasite related illness goes undiagnosed, which may lead to a skewed reporting on the incidences of parasitic illness (Dorny *et al.*, 2009). Depending on the reporting systems in different countries, an accurate estimate of the yearly incidence of food borne diseases is difficult and sometimes impossible. The prevalence of specific parasites in food supplies varies between countries and regions (Anantataphruti, 2001)

Use of sewage water to irrigate vegetables is a common practice in developing countries including Pakistan. It is estimated that at least 20 million hectares in 50 countries are irrigated with raw or partially treated wastewater (Dreschel *et al*., 2002). A small survey conducted by International

* Corresponding author: khanumbreen@yahoo.co.uk
0030-9923/2014/0005-1303 $ 8.00/0

Water Management Institute, in southern Punjab, Pakistan revealed that wastewater was being used without treatment for irrigation (Ensink *et al.*, 2004a,b). Eating raw vegetables is customary in Pakistan including many parts of the world. There are no reports from Pakistan about the contamination of vegetables by parasites. So keeping in view the importance of parasitic infections and their probable association with vegetables (especially raw ones) the study was designed to determine the prevalence of parasitic infection due to raw vegetables eaten in Lahore.

MATERIALS AND METHODS

Sample collection

A total of 500 samples of 10 different types of vegetables were taken from field, wholesale and retail markets of Lahore city during January-April, 2011.

The vegetable samples included beet (*Beta velgaris*), cabbage (*Brassica denceal*), carrot (*Davcus carota),* chili (*Capsicum annum*), corriander (*Corriandum sativum*), cucumber (*Cucumis sativa*), podina (*Mentha viridis*), radish (*Raphanus sativus*), tomato (*Lycopersicon esculentun*) and lettuce (*Lactuca sativa*). Out of total 500 samples, 100 were collected from 15 farms around the suburban areas of Lahore, 200 samples different shops in 2 major wholesale markets and 200 samples from retail markets and sellers.

Sampling from the retail markets and the green groceries was done randomly from different parts of Lahore including north, south and center of the city. At each market, samples were collected under normal purchase conditions from three randomly selected sellers. Samples were collected from the upper, middle and lower shelves of each seller. A minimum of three composite samples each containing one whole cabbage head, three bunches of lettuce and 250 - 500 g for other vegetables were taken. These vegetables were collected into sterile, labeled polythene bags and transported to the laboratory in the Department of Parasitology at University of Veterinary and Animal Sciences, Lahore within 3 h of collection for the examination and were studied on the same day, mean temperature ranging from 6-26°C. Spoiled, damaged and vegetables with excessive dirt were not collected.

Sample analysis

Sedimentation method

Samples of each vegetable (200-250 g) were washed in distilled water in a plastic container for the removal of the parasitic ova, larva or cysts. The suspension was strained through a sterile sieve to remove undesirable materials. The filtrate was centrifuged at 5000 rpm for 5 min (Gharavi *et al*., 2002) and the supernatant was discarded into the disinfectant jar. A drop of the pellet was examined under the microscope to investigate parasites using 10X and 40X objectives. The eggs/cysts were identified based on morphological details as described by Soulsby (1982).

Floatation method

Samples of each vegetable (200-250 g) were washed in distilled water in a plastic container for the removal of parasitic ova, larva or cysts. The suspension was strained through a sterile sieve to remove undesirable materials. The filtrate was centrifuged at 5000 rpm for 5 min (Gharavi *et al*., 2002). The supernatant was discarded into the disinfectant jar. The sediment obtained was resuspended in zinc sulphate floatation fluid and recentrifuged. The floatation fluid was examined under microscope using 10X and 40X objectives. The eggs/cysts were identified based on morphological details as described by Soulsby (1982).

Statistical analysis

Data analysis was done with the help of SPSS 16.0. The chi - square test was used to find out the association between categorical variables and a P-value <0.005 was considered as significant. High parasitic contamination was observed in leafy vegetables as compared to root and fruit vegetables. According to P-value ($P<0.005$) significant association were observed for parasitic detection with respect to the type of vegetables.

RESULTS

Of 500 samples examined, 156 samples were found contaminated. Out of contaminated, 81

samples were of leafy, 45 were of root vegetables and 30 samples were of fruit vegetables. In contaminated samples, twelve genera of parasites (helminths, protozoa and cestodes) were recorded in the study. Among parasites recovered, *Ascaris lumbricoides* was most common parasite (37.17%) found in the study. *Ascaris* was isolated in 58 out of 156 positive samples.

Table I shows the frequency of distribution of contamination for each vegetable. Lettuce was the most contaminated vegetable - 24 (48%) samples were contaminated with parasites, followed by cabbage 22 (44%), podina (mint) 21 (42%), carrot 17 (34%), radish 15 (30%), coriander 14 (28%), beet 13 (26%), cucumber 12 (24%), tomato 10 (20%) and chili 8 (16%). Carrot showed 34% contamination in the present study. The least number of parasites *viz.*, 12%, 10% and 8% were found on cucumber, tomato and chili, respectively.

Hookworm (*Ankylostoma deudenale, Necatrus americanis*) was the second most abundant (10.8%) parasite in the vegetables examined, followed by *Trichostrongyloides* species (8.9%) (*Haemonchus, Cooperia, Strongylus* sp. etc). The ova of this super family is hard to distinguish without culturing larvae, so they are put into one group *Trichostrongyloides* species. Other parasites recovered from the study are *Trichuris trichiura* (6.4%), *Entamoeba* spp. (*Entamoeba histolytica* and *Entamoeba dispar* (7.05%); cysts of both parasites are similar), *Giardia lamblia* (4.4%), *Enterobius vermicularis* (3.2%), *Hymenolepis nana* (5.7%), *Taenia* species (5.1%), *Fasciola* species (5.1%), *Toxocara* species (3.8%) and the least observed parasite was *Toxoplasma gondii* (1.9%). The overall prevalence of different parasites was estimated to be (31.2%).

DISCUSSION

In the present study, examination of vegetables revealed twelve genera of parasites. The overall prevalence of different parasites was estimated to be 31.2%. These results are supported by (Daryani *et al*., 2008) who reported the detection of intestinal parasites in 29% (13/45) of native garden vegetables consumed in Ardabil city, Iran. The study results are also consistent with research in Jos, Nigeria (Damen *et al*., 2007) which showed 36% of vegetables contaminated with parasites (Uga *et al*., 2009) from Hanoi and Gharavi *et al*. (2002) from Tehran reported that (35%) vegetables were contaminated with parasites, which are also in accordance with the present study.

The high rate of parasitic contamination observed in these studies might be due to poor hygienic and sanitary handling of vegetables, poor transport and packaging of vegetables, waste and contaminated water irrigation and washing and other conditions of cultivation (Orlandi *et al*., 2002). Table I shows the frequency distribution of contamination for each vegetable. Lettuce was the most contaminated vegetable; 24 (48%), followed by cabbage 22 (44%), podina (mint) 21 (42%), carrot 17 (34%), radish 15 (30%), coriander 14 (28%), beet 13 (26%), cucumber 12 (24%), tomato 10 (20%) and chili 8 (16%). The study results are similar to those of (Damen *et al*., 2007; Amoah *et al*., 2006).

The high contamination of cabbage and lettuce might be due to their broad leaves that provide large contact area with contaminated irrigation water and soil (Larkin *et al*., 1978). Cabbage, lettuce and other green 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 *et al*., 2011; Amoah *et al*., 2006; Kozn *et al*., 2005).

Furthermore, 42% of the mint samples followed by 28% of coriander were found contaminated in this study. These observations are supported by (Hajjami *et al*., 2013). The high contamination percentage observed for mint can be supported by its development near ground level, where contaminated water and soil are in direct contact with this herb, also the dense foliage of mint protects cysts and eggs of parasite from hostile environmental conditions. In addition, the overlapping leaves of this plant protect helminths' eggs from sunlight, desiccation and wind (Idrissa *et al*., 2010; Dssouli *et al*., 2006; Bouhoum and Amahmid, 2002; Stein and Schwartzbrod, 1990).

Carrot showed 34% of contamination followed by beet root (26%) in the present study. Similar results are reported by Hajjami *et al*. (2013),

Table I.- Prevalence of parasites and their frequency distribution in different vegetables.

Parasitic names	Leafy (n=200)				Root (n=150)			Fruit (n=150)			No.	Percent
	Cabbage (n=50)	Lettuce (n=50)	Podina (n=50)	Coriander (n=50)	Radish (n=50)	Beet (n=50)	Carrot (n=50)	Cucumber (n=50)	Chili (n=50)	Tomato (n=50)		
Trichuris	2	3	2	0	0	1	0	0	1	1	**10**	6.41*
Trichostrongyloides	2	0	2	1	2	2	2	2	1	0	**14**	8.97*
Toxoplasma	0	1	0	1	1	0	0	0	0	0	**3**	1.92
Toxocara	1	2	0	1	0	1	1	0	0	0	**6**	3.84
Taenia	1	1	1	1	1	1	1	1	0	0	**8**	5.12*
Hymenolepis	1	1	1	1	1	1	1	1	0	1	**9**	5.76*
Hook worm	3	3	3	1	2	0	1	1	2	1	**17**	10.89**
Giardia	1	1	2	0	1	1	1	0	0	0	**7**	4.48
Fasciola	1	1	1	1	0	1	1	1	0	1	**8**	5.12*
Enterobius	0	1	1	1	0	0	1	0	0	1	**5**	3.20
Entamoeba	2	2	0	1	1	0	1	1	2	1	**11**	7.05*
Ascaris	8	8	8	5	6	5	7	5	2	4	**58**	37.17**
Total	22	24	21	14	15	13	17	12	08	10	**156**	100

A significant difference (P<0.005) was observed among the prevalence of different parasites in vegetables, Chi-Square= 16.92

* : Significant (P<0.05)

** : Highly significant (P<0.001)

Damen *et al*. (2007) and Uneke (2007). This high contamination might be due to the specialized rough skin, the pits and crevices in this vegetable retain some dirt which may not be easily removed by slightwashing in the field or retail outlet.

The least number of parasites were found on cucumber (12%), tomato (10%) and chili (8%) in this study. These observations are in accordance with Uga *et al*. (2009) who reported that fruit vegetables (eggplant, cucumber, tomato, chili etc) were least contaminated (3%) as compared with leafy (31%) and root vegetables (17%). It is due to the fact that these vegetables have smooth skin which enhance the removal of parasite eggs when washed.

Of 500 samples examined, twelve genera of parasites (helminthes, protozoa and cestodes) were recorded in the study. Among parasites recovered, *Ascaris lumbricoides* was the most common. *Ascaris* was isolated in 58 out of 156 positive samples showing 37.17% contamination. This is consistent with studies reported from Vietnam (Uga *et al*., 2009) which showed 21%, contamination of vegetables with *Ascaris lumbricoides*. These findings are also supported by Anwar and Mckenry (2012) and Ensink *et al*. (2004a,b).

Hookworm (*Ankylostoma deudenale, Necatrus americanis*) was the second most abundant (10.8%) parasite in the vegetables examined. These results are inaccordance with Nuhu *et al*. (2012). *Trichostrongyloides* species (*Haemonchus, Cooperia, Strongylus* sp. etc.) were found (8.9%) followed by hookworm. The ova of this super family is hard to distinguish without culturing of larvae, so they are put into one group *Trichostrongyloides* species. Other parasites recovered from the study were *Trichuris trichiura* (6.4%), *Entamoeba* spp. (7.05%) (*Entamoeba histolytica* and *Entamoeba dispar* cysts of both parasites are similar), *Giardia lamblia* (4.4%), *Enterobius vermicularis* (3.2%), *Hymenolepis nana* (5.7%), *Taenia* species (5.1%), *Fasciola* species (5.1%), *Toxocara* species (3.8%) and the least observed parasite was *Toxoplasma gondii* (1.9%).

Giardia cysts were found on 4.4% of vegetable samples, which is consistent with study on vegetables in Costa Rica (Monge *et al*., 1996) which showed 5% contamination with *Giardia* cysts. Amoros *et al*. (2010) reported 52.6% *Giardia* in cabbage and lettuce. In this survey 5.1% of vegetables were contaminated with *Taenia* spp. eggs. Al-Megrin (2010) from Saudi Arabia and Abougrain *et al.* (2010) from Libya reported 19.7% and 22% of vegetables contaminated with eggs of *Taenia* spp., respectively.

In this study 3.8% contamination of vegetables was reported by *Toxocara* spp. eggs (Kozan *et al.*, 2005) also found 2.5% of *Toxocara* in unwashed samples of vegetables in Turkey. Hajjami *et al*. (2013) observed 9.1% lettuce, 11.1% mint, 16.1% parsley and coriander contaminated by eggs of *Toxocara* in Morocco. In the present study, 3.2% of positive samples were of *Enterobius*. Al-Shawa and Mwafy (2007) and Zahid *et al*.(2004) reported 2.5% and 1.3% positive samples of vegetables having *Enterobius vermicularis* in Ghaza and Muzaffarabad city respectively. *Trichuris trichuria* was present in 6.4% of all positive samples of vegetables. These results are supported by Nuhu *et al*. (2012) which reported 1.3% of *Trichuris* in lettuce in Nigeria.

The present study showed 1.9% of *Toxoplasma* cysts on vegetables, while Al-Megrin (2010) reported high prevalence of 6.6% *Toxoplasma gondii* on vegetables, collected from markets of Riyadh, Saudi Arabia. 5.1% of *Fasciola* was examined in this study, which is in close accordance with study in Iran (Daryani *et al*., 2008).

CONCLUSION/RECOMMENDTIONS

The results would seem to indicate that one of the important routes of parasitic infection is due to consumption of raw and unwashed vegetables. So, enlightenment programs for the on necessity of food sanitation and personal hygiene should be intensified. Furthermore it was also observed that parasitic counts was high in fresh vegetables, but it is important to mention that these vegetables did not show any visible signs of spoilage. Hence, the outward appearance may not be a good criterion to judge the parasitic quality of fruits and vegetables. All vegetables should be adequately washed before consumption and where possible, decontaminants should be included in the wash water.

Limitations of the study

The prevalence and concentration of the observed parasites may have been underrated due to the methods used in this study. Other potential routes of contamination such as harvesting procedures, contaminated environments during handling, transport and storage, or direct contamination from individuals involved in the production and processing of products were also not investigated.

ACKNOWLEDGEMENTS

The author appreciates the assistance of technical staffs of Parasitology Laboratory, University of Veterinary and animal Sciences, Lahore, for their cooperation during the study. The literature of subjects was provided by the Department of Zoology, Punjab University Lahore, Department of Botany, Quaid-e-Azam University Islamabad, University of Veterinary and Animal Sciences Lahore. The contribution of all these institutions of national importance of the fields of science and research is highly appreciated.

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(Received 5 April 2014, revised 22 June 2014)