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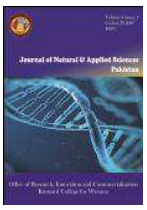

PREVALENCE OF PARASITIC CONTAMINATION IN LEAFY RAW VEGETABLE CONSUMED IN LAHORE, PAKISTAN

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Abstract

Fresh vegetables are considered as vital constitute of a healthy diet as they supply the body with essential supplements. But they also could be a potential source of parasitic infection among people. The present study was conducted to examine the level of parasitic contamination in freshly eaten uncooked vegetables sold in local vegetable markets of Lahore, Pakistan. Three vegetables was studied: cucumber, cabbage and lettuce. 50 samples of each vegetable was collected, comprising of 150 vegetable sample in total. The samples were analyzed through sedimentation and floatation method. Vegetables samples were examined using alight microscope. Overall prevalence of parasitic contamination among the sampled vegetable was 14%. Among the three, Lettuce was most contaminated, followed by cabbage and cucumber was found to be least contaminated. Parasite observed during the study include: *Entamoeba histolytica (*28.5%*), Balantidium coli* (14.28%)*, Fischoederius cobboldi (*14.28%*) Coccidia spp* (9.5%)*, Cooperia spp* (4.76%)*, Ostertagia circumcincta* (4.76%)*, Fischoederius cobboldi (*14.28%*), Diphyllobothrium latum (*4.76%*) and Toxora spp (*4.76%*).* Among the vegetable market and shop, most of the contamination was observed from vegetable collected from the big open central markets. Results showed that raw vegetables can act as carrier of parasites leading to parasitic infection among people. This shows that there is need to educate sellers and buyers to keep the good hygiene condition during growth, harvesting, transportation and washing of vegetables before eating.

Keywords

Parasite, prevalence, contamination, leafy vegetables, illness, infection.

1. Introduction

Vegetables are vital source of the essential nutrient required for human body. They are an important component of healthy balanced diet. They provide a number of vitamins (A, E and C), nutrients, and minerals including fiber, folate, iron, and potassium (Mohamed , Siddig , Elaagip, Edris AMM & Nasir, 2016). Intake of vegetable makes a person less liable to liver damage, cancer, cardiovascular diseases and strokes and other serious illness. Due to increasing issue of obesity and its related diseases, people are turning more towards eating fresh raw vegetables because of their low calories and nutrition values. As they are not cooked so nutrition value does

not decreases due to heating (Fallah, Kheirabadi , Shirvani , Siavash and Dehkordi, 2012).

Along with carrying nutrients, raw vegetables can act as vectors for the transmission of parasitic egg, cysts and larva commonly of protozoa and helminthes. Leafy green vegetables, Lettuce and cabbage, have highest parasitic contamination. With increasing intake of these vegetables, many food-borne illness has increased (Fawzi, Sahin , Ibrahim and Shehata, 2004). There is an estimation that 300 million severe illness are caused by parasitic infection with approximately causing 200,000 deaths in economically developing countries (Nazemi , Raei , Amiri and Chaman, 2004).

Contamination is not only done at the production site of vegetable but it is important to know that improper handling at distribution, packing and selling sites are equally responsible for transmitting parasites to the vegetables (Drechsel P, Campilan D, and Jocker, 2001).

With increasing demand of fresh vegetables, the availability of fresh water for irrigation of vegetables has decreased. Other factors which have led to the use of waste water to be used for irrigation include the poor farmers are provided limited access to clean water. Fertilizers are so costly that farmers prefer to use manure for increasing the fertility which is one of the major reason for parasitic contamination in vegetables (Ofori , Abaidoo , Danso and Klank, 2009). Vegetables, especially the leafy raw vegetables use in salads, are reported to be contaminated with intestinal parasites such as Ascaris lumbricoides, Toxocara spp, Fasciola hepatica, Trichostrongylus spp, Echinococcus spp, Trichuris spp, Enterobius vermicularis, Hymenolepis nana, , Toxocara.spp, Strongyloides stercoralis, Taenia spp, Blastocystis hominis, Giardia lamblia, Entamoeba spp, Iodamoeba butschlii and Cryptosporidium parvum. In general, parasitic illness leads to nausea, stomach pain, vomiting, fever, weight loss and dehydration. Vegetables become available to pathogens during their growth, collection, harvesting, processing, and transportation, in shop's shelves and even at homes. Vegetables have become a route for parasitic contamination in human because of many unhygienic and uncontrolled practices (Mafiana , Sam-Wobo, and Akinsete , 2000).

Giardia lamblia, Ascaris lumbricodes and Entamoeba histolytica play the largest role in such parasitic contamination. It is known that Ameobiasis is the cause of about 450 million infections each year in developing world, causing about 50 million and 100,000 deaths (Ukoli, 1990). Giadiasis is more commonly found in children and has a worldwide prevalence of about 1– 30%. Ascariasis is the most commonly found nematode of man especially in tropical Africa (Bekele, Biresaw and Yohannes, 2017). According to a research, eggs of Ascaris can live for six years (Dreschsel, Blumenthal and Keraita, 2001). According to an estimation in 50 countries, 20 million hectares of land is irrigated using untreated waste water. Industries do not treat their waste water and just release their waste in to the rivers and seas (Khan , Mumtaz, Bibi and Afzal , 2017).

Organic fertilizer (compost, animal manure, sewage) are not treated and processed before applying on agricultural land, especially in developing countries (where farmers are not much educated and also government does not facilitate them much). As animal manure and human fecal matter contains many parasites. These parasites should be removed from the animal manure to make it safe to apply on agricultural lands otherwise these organic fertilizers can also become a factors in contaminating vegetables with parasites (Ishaku , Ishakeku and Agwale, 2013).

Another way of getting parasitic illness is because of improper and unhygienic handling of the vegetables by food handlers which can transmit parasites to their customer. The vegetables are present not kept in very hygienic ways in local shops. The local shop keepers and vendors sprinkle water on vegetables to keep them soft and fresh. But they do not use clean water. Mostly water bucket is not closed, and they even use this water for cleaning their hands. This water also become a source of parasite on vegetables. Sometimes, even at homes, vegetables are not washed properly before eating (Balark et al, 2016).

Parasitic contamination is not only found in developing nations buts also in developed countries as well. But the, ratio of parasitic infection is less in developed countries The most probable answer to this is increasing trade and transport of refrigerated food, migration of people infected with parasites to the developed countries, more trend of raw food like sushi. Most of the time parasitic infection goes

undiagnosed and especially when it has similar symptoms to viral or bacterial infection. Also there are inadequate ways to detect and treat parasitic contamination and illness that is why there is very less number of reports found on parasitic illness. An accurate estimation of incidence of parasitic infection is not easy even sometimes not possible. The presence of specific parasites in food differ from region to region (Andoh, 2006). It has been estimated that humans harbor about 300 species of parasitic worms and over 70 species of protozoa (Doyle, 2003).

To remain safe from these parasitic infection it is important to eat vegetables and fruit after proper washing, do not swallow water from the swimming pools, lakes and rivers. Wash hands properly after using uncooked food or come in contact with feces, especially those are pet lovers. (Marcin, 2016).

2. Methodology

2.1 Study Area

The study was conducted in Lahore, the capital city of Punjab province, Pakistan. It is a densely populated city with a population of 9,508,000. It covers as area 1772 km2. There are not much agricultural land inside the city but are in the outskirts of Lahore. Most of the vegetable found in Lahore come from other cities like Sialkot, Gujranwala, Okara, and Multan.

2.2 Sampling Locations

Sampling is done from three type of vegetable markets: Open Markets (Mandi), retail markets, and Vendors during January to April 2018. Two open market were targeted: one in Badami bagh one in Gajjumatta.

2.3 Collection of Samples

Samples include three commonly used vegetables in Salad i.e., Cucumber (Cucumis Sativus), Cabbage (Brassica oleracea), Lettuce (Lactuca sativa). The samples were collected randomly from these markets. Only the vegetable that were not spoiled were collected.The vegetables were collected in polyethylene bags (Labelled with name of market, number of samples and date) and then taken to Biotechnology Laboratory of Kinnaird College for Women, for processing and analysis.

2.4 Sample Analysis

2.4.1 Sedimentation Method

Each vegetable was weighted (200-250g) using a weighing balance. Individual leaves of lettuce was used and cabbage was divided into four parts.Then they were put in the plastic container and beaker.100ml of Saline (10%) was added to each sample for washing.Then the washing samples were then left for over-night sedimentation. Next day, washing samples were passed from sieve to remove undesirable materials. The filtrate is then poured into 15ml Falcon tube and centrifuge at 2000 rpm for 15 minutes.The supernatant is discarded and sediment was then examined by placing a drop of sediment in the center of a clean grease-free glass slides.Glass slide is examined under Light microscope using 10X and 40X objectives.Identification of egg/cyst was done with the help of morphological details given in Soulsby (Soulsby, 1982).

2.4.2 Floatation Method

After sedimentation method, the washing sample left will be used for this method. The filtrate is poured into 15ml falcon tube and centrifuge at 2000 rpm for 15 minutes.The supernatant was discarded The sediment formed was re-suspended into Zinc Sulphate floatation fluid (200µl for each sample).This mixture containing sediment and Zinc Sulphate floatation fluid was then transferred into Eppendorf tube . It is then again centrifuge at 2000 rpm for 15 minutes.Then, using a dropper, only the solution from the above the fluid (Floatation fluid) is transferred to a clean glass slide. Glass slide was observed under light microscope using 10X and 40X objective. Identification of egg/cyst was done with the help of morphological details given in Soulsby (Soulsby, 1982).

2.5 Statistical Analysis

Data analysis was carried out by using Chi-square test on SPSS 21.0 to find out association between the parasitic contamination and vegetables found in different markets. P value< 0.005 was considered significant.

3. Results

Out of total 150 vegetable samples, which comprises of 50 cucumbers, cabbage and lettuce each, 21 (14%) vegetable were found to be

contaminated with parasite. Parasites found were belonging to different genera. Among the three vegetable examined, most contaminated was found to be Lettuce (12/50), and followed by Cabbage (7/50) and Cucumber (2/50). Among parasites, Entamoeba histolytica was most prevalent parasite (33.3%), found in sample of cucumber (1/50), in 2 samples of cabbage (2/50) and 3 samples of lettuce (3/50).

Table 1. Prevalence of parasites in the vegetables collected during sampling

Parasite name	Vegetable name			No. of parasite found	Percent (%)
	Cucumber	Cabbage	Lettuce		
Entamoeba histolytica	1	2	3	6	28.5%
Balantidium coli	0	2	1	3	14.28%
Fasciola hepatica	1	1	1	3	14.28%
Fischoederius cobboldi	0	1	2	3	14.28%
Coccidia spp	0	0	2	2	9.5%
Cooperia spp	0	0	1	1	4.76%
Ostertagia circumcincta	0	0	1	1	4.76%
Toxora spp.	0	0	1	1	4.76%
Diphyllobothrium latum	0	1	0	1	4.76%
Total	2	7	12	21	100%

Table 1 show the frequency and percentage of contamination found in vegetables. Balantidium coli, Fasciola hepatica, Fischoederius cobboldi was the second most abundant parasite found with a percentage of 14.28%. Other parasite found were *Coccidia spp (9.5%), Cooperia spp (4.76%),Ostertagia circumcincta (4.76%),Toxora spp (4.76%),Diphyllobothrium latum (4.76%).*

Table 2. Prevalence of contaminated cucumber, cabbage and lettuce in different vegetable markets/ shops

Sr. No.	Type of vegetable market	Location of market	No. of contaminated cucumber out of total no. of sample taken of cucumber	No. of contaminated cabbage out of total no. of sample taken of cabbage	No. of Contaminated lettuce out of total no. of sample taken of Lettuce
1	Big Open Central Market	Badami Bagh	1/12 (8.3%)	3/10(30%)	3/10(30%)
2	Big open central market	Gajjumatta Kanamandi	1/10 (10%)	2/10(20%)	4/10(40%)
3	A.H Brother	Askari 9	0/8(0%)	0/4 (0%)	1/4 (25%)
4	Vegetable shop	Saddar bazar	0/8(0%)	1/8(12.5%)	1/8(12.5)
5	Vegetable shop	Jaurypul	0/5(0%)	0/8 (0%)	1/8(12.5)
6	Vendor	Dharmpura	0/5(0%)	0/5 (0%)	1/5(20%)
7	Vendor	Saddar bazaar	0/2(0%)	1/5 (20%)	1/5(20%)
8	Total	7	2/50(4%)	7/50(14%)	12/50(24%)

Table 2 shows the comparative analysis of prevalence of contaminated cucumber, cabbage and lettuce in different vegetable markets/ shops. Highest rate of contaminated lettuce samples were found from the two big central markets in Lahore, In Gajjumata and one in Badami Bagh. Following tables shows prevalence of contaminated cucumber, cabbage and lettuce with respective to type of their vegetable market.

4. Discussion

In the present study, three type of commonly used raw vegetables in Lahore were examined for the parasitic contamination. Over all prevalence of parasite found was estimated to be 14%. Different prevalence had been reported by different researcher. Fortunately, it is lower from studies conducted in Iran (Rostami et al, 2016), Nigeria (Damen, Banwat, Egah and Allanana, 2007), Vietnam (Tram and Dalsgaard ,2014), Brazil (Luz, 2017), and Jordan (Ismail, 2016). The result of present study is supported by Mohammad et al. (2017) who observed prevalence of 13.5%.

Entamoeba hstolytica (28.5%) was found to be most prevalent parasite in the present study. This result is supported by Piranshahi et al. (2017) who reported 5.34% of Entamoeba spp which is higher than of other parasite examined in his report. Balarak et al. (2014) reported 11.4% of Entamoeba hstolytica which is highest among other pathogenic protozoan he examined. In all the previous study Entamoeba hstolytica was found with different percentages ranging from 5.3% to 14.0. This percentage of Entamoeba spp indicate a substantial amount of fecal contamination in the vegetable. Major reason is

the use of animal dung as manure for the crops. It clearly indicate that vegetable sold in Lahore are irrigated with wastewater or untreated water as these parasite are common among people who do not have access to clean drinking water and have inadequate sanitation system.

Lettuce was found to be most contaminated with parasites. This is supported by studies of Duedu et al. (2014), Ismail (2016), and Silva (1995). Most of vendors and even the shopkeeper do not wash lettuce properly, unlike other vegetable such as cucumber, because leaves of lettuce are soft and fragile. This makes Lettuce a potential source of parasitic illness in human.

Cabbage was contaminated with 7 parasites, followed by lettuce, in present study. This result was supported by Haq et al. (2014). The reason for the high rate of contamination in lettuce and cabbage is due to their broad leaves which gives more surface area for the contaminants to attach on the vegetable. Along with this, they have rough surface which make it easy for parasite to attach. In addition, leaves of lettuce are soft and wide. In contrast, Cucumber having smooth shiny surface make less easy for parasite to attach and survive. That is why cucumber is least contaminated in the present study.

In the present study, most of the contaminated vegetables examined were collected from Big open central market, followed by vendors and local shops (in markets). It was observed in Big open central market (Gajjumata Kana mandi in Gajjumata and a Lahore mandi in Badami Bagh) during collection of vegetable that vegetables were placed in unclean basket, with dirt on it ,and some of sellers had placed their vegetables on a sheet of cloth. There was no washing of vegetable. Shop keepers and vendors also mat get their vegetables from these big open market. But it was observed that vendors and shop keepers sprinkle water on vegetables to keep them fresh. The hygienic condition of the shopkeepers was better than of the vendors and sellers in open market. According to the study of Duedu et al. (2014), who compares vegetables for parasitic contamination from super market and big open market, reported to use saline for washing vegetables. This study supports the present study in which the samples were washed with physiological saline.

The results show that consumption of raw and unwashed vegetables might be the one of the important routes of parasitic infection. The outward appearance may not be a good criterion to judge the parasitic quality of vegetables. All vegetables should be adequately washed before consumption and where possible, decontaminants should be included in the wash water.

5. Conflict Of Interest

The authors of this work declare that they have no conflict of interest

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