RESEARCH PAPER | Zoology | Volume : 6 | Issue : 2 | FEBRUARY 2016 | ISSN - 2249-555X

Assessment of Parasitic Protozoan Infestation on Commonly Consumed Raw Vegetables and Their Sources of Contamination

KEYWORDS: Food borne protozoan infections, Parasitic protozoans, Vegetables.

Sameer Sood
M.Sc. student, Department of Zoology, Punjab Agricultural University, Ludhiana – 141004, Punjab, India

D.K. Kocher
Associate Professor, Department of Zoology, Punjab Agricultural University, Ludhiana – 141004, Punjab, India

ABSTRACT *Vegetables are commonly eaten raw or partially cooked and are known to serve as vehicle of human diseases, especially those related with parasitic protozoans. Vegetable samples collected during different seasons from the selected study areas viz; eight vegetable fields, four local vegetable markets and three supermarkets during the year 2015 were processed for extraction of parasitic protozoans (cysts/oocysts/sporozoites). Different sources of contamination (irrigation water, soil and washing water) were also tested to check the transfer of parasitic protozoans through these sources. Incidence of parasitic protozoans on commonly consumed raw vegetables was found to be highest during rainy season (35.18-80.56%) followed by winter (33.33-63.89%) and least during summer season (7.41-43.06%), irrespective of the site of collection. Maximum protozoan contamination of soil (63.41%), irrigation water (75.00%) and washing water (88.89%) was found during rainy season followed by winter (41.46-83.33%) and summer (20.83-72.22%). Vegetable samples collected from local markets showed higher parasitic protozoan infestation (80.56%) during all the seasons in comparison to village fields (64.23%) and supermarkets (35.18%). Leafy vegetables were found to be maximally contaminated (56.86-73.53%) with parasitic protozoans followed by root/modified stem vegetables (11.11-46.67%) and lowest in fruit vegetables (20.37-27.78%).*

Introduction

Vegetables are an important source of nourishment containing carbohydrates, proteins, vitamins, minerals as well as trace elements (Itanna 2002). Fruits and vegetables can become contaminated with pathogens capable of causing human diseases while still on the plant in fields or orchards, or during harvesting, transport, processing, distribution and marketing, or in the home (WHO 1998). The extent of contamination depends on several factors that include use of untreated wastewater and water supplies contaminated with sewage for irrigation (Kozan*et al* 2007). The consumption of raw vegetables without proper washing is an important route in the transmission of parasitic diseases and there has been an increase in the number of reported cases of food-borne illness linked to fresh vegetables (Said 2012).It has been estimated that humans harbour about 300 species of parasites and major are acquired from contaminated raw fruits and vegetables (Doyle 2003).

Several surveys in different parts of the world showed that commonly consumed raw vegetables can be agents for transmission of cysts and oocysts of protozoans like *Giardia, Entamoeba,Cryptosporidium, Cyclospora, Taxoplasma* and *Isospora,* thus playing a major epidemiological role in the transmission of parasitic food-borne diseases (Olyaei and Hajivandi 2013). Evidence for faecal contamination of surface and ground water is provided by the detection of enteric pathogens in these which has resulted in the continued occurrence of outbreaks of waterborne disease (Morteza 2001). Concurrent cleaning and disinfection of the environment is expected to increase the effectiveness of treatment by reducing the parasite burden (Thompson and Monis 2012). Acceptable removal requires well designed and operated systems. Membrane filtration processes that provide a direct physical barrier may represent a viable alternative for the effective removal of oocysts from water. Owing to the exceptional resistance of the oocysts to disinfectants, this cannot be relied upon as an index for the presence/absence of oocysts in drinking-water supplies (Corso 2003). The oocysts are resistant to disinfection and are not inactivated by chlorination practices generally applied in the production of drinking-water. Control measures that can be applied to manage potential risk from pathogens include prevention of source water contamination by human waste, followed by adequate treatment and protection of water during distribution (Said 2012).

Materials and methods

Selection of study areas and collection of vegetable, water and soil samples

In the present study, eight vegetable fields (Narangwal, Balloke, Baba Deep Singh Nagar, Bounkardogran, Mangat, Birmi, Dhanoa and Sant Vihar) near Buddha Nullah (a seasonal water stream contaminated with domestic and industrial sewages of the city), four local vegetable markets (Chhoti Haibowal market, Badi Haibowal market, Dairy Complex market and Chander Nagar market) and three supermarkets (Easyday market, Reliance market and Metro market) of Ludhiana, Punjab (30.91° North and 75.85° East) were selected to check the incidence of parasitic protozoans (cysts/oocysts/sporozoites). Samples of vegetables from fields, local markets and supermarkets (N=753), soil (N=125), irrigation water (N=72) and washing water (N=162) were collected throughout the year 2015 according to the availability of crops in winter, summer and rainy seasons (three times/season).

Extraction and identification of parasitic protozoans

In laboratory, the vegetables were chopped in 4-5 cm pieces and ~250 gm of each vegetable was immersed immediately in tap water inside a sink and left approximately 6–7 min for sedimentation of mud and dust. Vegetable pieces were gently collected and were put in a plastic container. Each vegetable sample was eluted

Volume : 6 | Issue : 2 | FEBRUARY 2016 | ISSN - 2249-555X

for 30 min in 1 L of sterile phosphate-buffered saline to which 50 ml of 0.01% tween 80 was added. The eluent was filtered through gauze and then dispensed into clean centrifuge tubes and centrifuged at 2000 rpm for 30 min. The supernatant was discarded and the pellet was washed with Tween 80 by centrifugation. The pellet was then agitated gently with hands in physiological saline solution for the homogeneous distribution of the cysts, oocysts and sporozoites in the residue (Said 2012). Each water sample (irrigation water and washing water) was passed through a filter paper and then the filter paper was rinsed with 0.01% tween-80. The filter was centrifuged at 3000 rpm for 5 minutes. The pellet was suspended for further analysis (Graczyk and Fried 2007) i.e. to check the presence of protozoan parasites by microscopy. Each soil sample (250 gm) was put in 1000 ml water. The mixture was passed through a gauze. The solution was centrifuged at 2500 rpm for 5 minutes. The pellet was suspended for further analysis (Olyaei and Hajivandi 2013) i.e. to check the presence of protozoan parasites by microscopy. Different types of parasitic protozoans were identified on the basis of standard morphological keys provided by Gardiner *et al* (2012). Data was statistically analysed by using Chi-square test.

Results and Discussion

Seasonal incidence of parasitic protozoans on commonly consumed raw vegetables collected during different seasons from different sites

During every season (either winter, summer or rainy), the highest incidence of parasitic protozoans was observed in the vegetable samples collected from local markets followed by that of village field samples and least from supermarkets.The highest incidence of parasitic protozoans was observed in the vegetable samples collected from local markets during rainy season was 80.56% followed by winter season (63.89%) and summer season (43.06%). The incidence in vegetables collected from vegetable fields was 64.23% during rainy season followed by winter season (41.46%) and summer season (13.95%). Vegetables collected from supermarkets showed incidence of parasitic protozoans @ 35.18, 33.33 and 7.41% during rainy, winter and summer season respectively (Fig. 1). High level of contamination of vegetables with parasitic protozoans during rainy season may be because of the favourable conditions like high humidity and moisture available during this season to parasitic protozoans to sustain in the environment. During winter season, the low temperature and high moisture also favours their survival, but in summer the parasitic protozoans mostly die-off due to high temperature, rendering a comparatively low incidence (Said 2012). The observations of present study were in agreement of the results given by Dawson *et al* (2005) who reported that recovery rate of cysts and sporozoites from vegetables is related to seasons i.e. more cysts and sporozoites in the rainy season (64%) than in the dry season (12%). The most likely hypothesis of contamination of vegetables with protozoan load may be due to pre-harvest contaminants like manure, manure compost, sewage sludge, irrigation water, runoff water from livestock operations or directly from wild and domestic animals. These potential contamination events are all plausible and consistent with the assumption that the level of contamination must have been high (Ali and Ameen 2013). Nirmi (2012) has also observed high prevalence of natural infection in leafy vegetables with cryptosporidiosis and cyclosporiasis in the rainy season compared to other seasons.

Contamination of vegetables with respect to sources of contamination

The parasitic protozoan contamination in field vegetables was found to be maximum during rainy season (64.23%) which might be due to the high level of protozoan infestation in field soil (63.41%) and irrigation water (75.00%) but the vegetables sold in the local markets showed 80.56% parasitic infestation which was found to be higher than that of the field vegetables during this season. It may be due to the usage of contaminated washing water used for washing of vegetables before selling as this water showed 88.89% infestation of these protozoans. During winter and summer seasons also, vegetables being sold in the local markets were more contaminated as compared to the field vegetables because of their washing with highly contaminated water. Post-harvest faecal contamination of vegetables may also occur during handling, transport and splashing the vegetables with contaminated water in order to keep vegetables fresh (Andoh 2006). The high occurrence of these parasites reflects a high rate of human infection (Gibson 1994). The lowest level of protozoan infestation in vegetables collected from supermarkets (35.18%) revealed their further washing with clean water and their maintenance under hygienic conditions (Table 1).

Incidence of parasitic protozoans on different types of vegetables collected from different sites

The comparative analysis of parasitic protozoans on different types of vegetables showed their highest incidence in leafy vegetables followed by root/modified stem vegetables and then fruit vegetables. The incidence of parasitic protozoans in leafy vegetables was found to be 73.53, 60.42 and 56.86% in coriander, mint and cabbage. Among the root vegetables, the incidence of parasitic protozoans was 46.67 and 37.63% in carrot and radish respectively, while onion, the type of modified stem vegetable showed less protozoan incidence (11.11 %). Among the fruit vegetables, the incidence of parasitic protozoans was 27.78, 20.37 and 24.56% in cucumber, armenian cucumber and tomato (Table 2). The statistical analysis of data showed that protozoan infestation was found to be significantly related to each other at $p \leq 0.01$. Higher level of contamination of parasitic protozoans in green leafy vegetables is because of the fact that such vegetables have uneven surface and makes protozoan (cysts/oocysts/sporozoites) attached to the surface of vegetables more easily, either in the farm or during washing with contaminated water. On the other hand, vegetables with smooth surface like tomato has the least incidence as their smooth surface reduces the rate of parasitic attachment (Damen *et al* 2007). These findings may have important implications for global food safety and emphasize the importance of raw vegetables in threatening public health by transmission of intestinal parasites to humans (Kniel *et al* 2002).

Acknowledgement:

Authors are thankful to Head, Department of Zoology, Punjab Agricultural University, Ludhiana for providing the financial assistance to carry out this research work

Volume : 6 | Issue : 2 | FEBRUARY 2016 | ISSN - 2249-555X

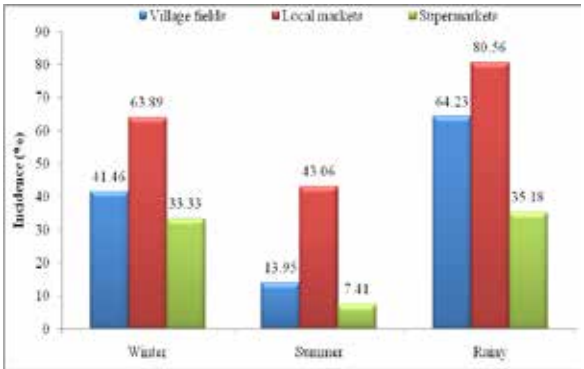

Fig. 1: Seasonal incidence of parasitic protozoans on commonly consumed raw vegetables collected from different sites

Table 1: Incidence of parasitic protozoans in samples of irrigation water, field soil, washing water and vegetables collected during different seasons

Season	Incidence of parasitic protozoans (%)						
	Irrigation water	Soil	Village fields	Washing water	Local markets	Super markets	χ^2 value
Winter	41.67	41.46	41.46	83.33	63.89	33.33	3.54*
Summer	20.83	23.26	13.95	72.22	43.06	7.41	
Rainy	75.00	63.41	64.23	88.89	80.56	35.18	

* represents χ^2- value among irrigation water, soil, village fields, washing water and local markets which is less than table value at 2df ($p \leq 0.01$) i.e. 4.61 indicating that results are significantly related with each other.

Table 2: Incidence of parasitic protozoans on different types of commonly consumed raw vegetables collected from different study areas

Types of vegetables	Vegetables	Village Fields		Local Markets (n=24)	Super Markets (n=18)	Total number of positive samples (% Incidence)	χ^2 value
		No. of samples	No. of positive samples (% Incidence)	No. of positive samples (% Incidence)	No. of positive samples (% Incidence)		
Root / Modified stem vegetables	Carrot (N=93)	51	17 (33.33)	16 (66.67)	2 (11.11)	35 (37.63)	22.35*
	Radish (N=105)	63	27 (42.86)	19 (79.17)	3 (16.67)	49 (46.67)	
	Onion (N=81)	39	3 (7.69)	6 (25.00)	0 (0.00)	9 (11.11)	
Leafy vegetables	Coriander (N=102)	60	39 (65.00)	24 (100.00)	12 (66.67)	75 (73.53)	
	Cabbage (N=51)	9	2 (22.22)	20 (83.33)	7 (58.33)	29 (56.86)	
	Mint (N=96)	54	30 (55.56)	19 (79.17)	9 (50.00)	58 (60.42)	
Fruit vegetables	American cucumber (N=54)	12	2 (16.67)	6 (25.00)	3 (16.67)	11 (20.37)	
	Cucumber (N=54)	12	1 (8.33)	10 (41.67)	4 (22.22)	15 (27.78)	
	Tomato (N=114)	72	12 (16.67)	15 (62.50)	1 (5.56)	28 (24.56)	

n represents the number of vegetable samples taken from village fields, local markets and supermarkets each time during the season.

* represents χ^2- value among village fields which is more than table value at 8df ($p \leq 0.01$) i.e. 13.36 indicating that results are significantly different from each other.

REFERENCE Ali, A S. & Ameen, K G. (2013). An epidemiological study of parasitic infections in stool specimen and correlation with waste water in Baghdad. J Duhok Univ 13: 63-67. | Andoh (2006). Outbreak of Cyclosporiasis associated with imported raspberries, Philadelphia, Pennsylvania.Emerg Infect Dis 8(8): 783–88. | Corso, P A. (2003). Parasites and the food supply. Food Technol 56: 72–81. | Damen, S E. Gerba, C P. & Pepper, I L. (2007). Confirmation of the human pathogenic Microsporidia Enterocytozoon bieneusi, Encephalitozoon intestinalis and Vittaforma corneae in water. Appl Environ Microbiol 64: 3332–35. | Dawson, D J. Samuel, C M.Scrannage, V. & Atherton, C J. (2005). Survival of Cryptosporidium species in environments relevant to foods and beverages. J Appl Microbiol 96: 1222–29. | Doyle, M E. (2003). Food-borne Parasites. A review of scientific literature, (pp. 1-28). Food Research Institute, University of Wisconsin, Madison. | Gardiner, J. Geerts, S. Madeline, M. Ballandonne, C. & Barbier, D. (2012).The use of standard keys for identification of parasites. Vet Res 33: 575–97. | Gibson, G H. (1994). Giardia duodenalis cyst survival in cattle slurry. Vet Parasitol 184(2): 330-34. | Graczyk, T K. & Fried, R. (2007). Detection of Cryptosporidium oocysts and Giardia cysts in the tissues of eastern oysters (Crassostrea virginica) carrying principal oyster infectious diseases. J Parasitol 84: 1039-42. | Itanna, F. (2002). Metals in leafy vegetables grown in Addis Ababa and toxicological implication. Ethiopian J Health Dev 16: 295-302. | Kniel, L. Robertson, L J. & Gjerde, B. (2002). Occurrence of parasites on fruits and vegetables in Norway. J Food Prot 64: 1793-1798. | Kozan, E. Sevimi, F K. Kose, M. Eserm, M. & Cicek, H. (2007). Examination of helminth contaminated wastewaters used for agricultural purpose in Afyonkarahisar. Turk Parasitol Derg 31: 197-200. | Morteza, M. (2001). Prevalence of intestinal parasites in selected vegetables at major public markets in Metro Manila, Philippines. Asian Pac J Trop Med 2: 37-39. | Nirmi, H. (2012).Control of helminth contamination of raw vegetables by washing. Vector Borne Zoonotic Dis 11(2): 189–91. | Olyaei, P A. & Hajivandi, K A. (2013). Extraction-free, filter-based template preparation for rapid and sensitive PCR detection of pathogenic parasitic protozoa. J Clin Microbiol 38(6): 2271–77. | Said (2012). Detection of parasites in commonly consumed raw vegetables. Alexandria J Med 48: 345-52. | Thompson, A H. & Monis, E B. (2012). Helminth eggs in raw and treated wastewater in the Islamic Republic of Iran. East Mediterr Health 12(1): 276-89. | WHO (1998). Guidelines for food borne diseases. (First edition) World Health Organization, Geneva. ISBN: 9235469855. |