

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.508.085>

A Comparative Study of the Occurrence of *Cryptosporidium parvum* oocysts found on Fresh Fruits and Vegetables Sold in Supermarkets and Open-aired Markets

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A B S T R A C T

Keywords

Vegetables and
fruits,
Cryptosporidium
oocysts,
Food safety.

Article Info

Accepted:
28 June 2016
Available Online:
10 August 2016

Human cryptosporidiosis has emerged as an important gastrointestinal infection in the 1990s as a result of the ingestion of mainly contaminated water and to a lesser extent food stuffs containing the protozoan parasite, *Cryptosporidium parvum*. Fresh vegetables and fruits are an important part of a healthy diet and Consuming raw vegetables offers essential nutrients that one may not get when such vegetables are usually cooked. However, eating them raw may pose a great risk for transmissions of pathogens. Such risks may be influenced by the sources of the vegetables and washing techniques used. No previous surveys have been conducted to evaluate the prevalence of *Cryptosporidium parvum* contamination of vegetables in Egypt. The aim of the study was to compare the prevalence and diversity of *cryptosporidium* associated with vegetables and fruits sold at the two types of markets and compare effectiveness of various washing techniques. The result was Contamination of vegetables sold at the open-aired markets was about ten-times that of the supermarkets.

Introduction

In recent years, there has been an increase in the number of reported cases of food-borne illnesses linked to consuming fresh vegetables. The consumption of raw vegetables plays a major epidemiological role in the transmission of parasitic food-borne diseases. Food normally becomes a potential source of human infection by contamination, during production, collection, transport and preparation or during processing. The sources of zoonotic contamination are usually faeces, faecally contaminated soil or water (Slifko *et al.*,

2000; Daryani *et al.*, 2008; Damen *et al.*, 2007). Cryptosporidiosis is a nationally notifiable gastrointestinal illness caused by chlorine-tolerant protozoa of the genus *Cryptosporidium*. Cryptosporidiosis is a major public health problem in both developing and developed countries which caused by *Cryptosporidium parvum*. It is a most common parasite and can contaminate vegetables and fruits if these vegetables are irrigated with contaminated water which is defecated by both human and animal due to poor hygienic condition of the environment

(McEvoy *et al.*, 2003). Cryptosporidiosis has been incriminated as the most frequent microbial cause of diarrhoea accompanied by pain and abdominal colic and a noticeable loss of weight. Cattle have been seen to be an important source of zoonotic cryptosporidiosis since 1980's (Guerrant, 1997). *Cryptosporidium* infection is transmitted by the faecale-oral route and results from the ingestion of *Cryptosporidium* oocysts through faecally contaminated water or food or through direct person-to-person or animal-to-person contact (Yoder *et al.*, 2007).

Cryptosporidium has also become a well-known cause of opportunistic infections among AIDS patients and of gastrointestinal disease outbreaks. Water and the food-chain have been reported as the main sources of outbreaks of diarrhea and other food-borne illnesses (Ali *et al.*, 2006). Though cooking at high temperatures are expected to kill most pathogens, this method may not apply to fresh vegetables which are rather eaten raw. Freshly eaten vegetables are a major source of nutrients like vitamins (vitamin B-complex, vitamin-C, vitamin A, and vitamin K) and minerals (calcium, magnesium, potassium, iron, and beta-carotene) as well as dietary fibre. They protect the body against infectious diseases and serve as phytochemicals which function as antioxidants and anti-inflammatory agents reducing the risk of cardiovascular diseases, stroke and certain cancers (Ali *et al.*, 2000; Ortega *et al.*, 1997). They also help prevent constipation, hemorrhoids, and rectal fissures among others. Foodborne outbreaks of cryptosporidiosis have been reported since 1980s and have been identified from several commodities, mostly fruits, vegetables, shellfish (McEvoy *et al.*, 2007). These products are usually eaten raw and there is a concern that food may be a vehicle of transmission of *Cryptosporidium*. The

main route of transmission of parasites diseases is the eating of raw vegetables without proper washing. Outbreaks of intestinal parasitic infections have been reported from developed and poor countries linked to raw vegetables and fruits (Ortega *et al.*, 1997; Ortega *et al.*, 1993). Raw vegetables may be also contaminated with *Cryptosporidium* oocysts during the post-harvest handling and processing. The degree of contamination of fresh vegetables mostly depends on several factors such as use of untreated waste water and water supplies contaminated with sewage for irrigation, and hygienic conditions of preparation in food service or home settings (Amoah *et al.*, 2007; Beuchat, 2002; Carreno *et al.*, 1999).

Egypt is an agricultural country where 80% people are associated with agriculture and almost all are involved with large or small scale vegetables farming. Huge amount of vegetables and fruits are produced countrywide every year. During harvesting and marketing, the uses of river, pond and contaminated sewage water are most common practices for irrigation of vegetables in Egypt. In addition, the poor hygienic condition of Egypt's environment which is mostly because of defecation by both animals and human can pollute water sources.

When such polluted water is used to irrigate raw vegetables and fruits can become contaminated with *Cryptosporidium* oocysts. In Egypt there are lots of markets countrywide where vegetables and fruits are sold and consumed. Therefore the main objective of this study is to determine the rate of contamination of vegetables and fruits with *Cryptosporidium* oocysts which will aware the people on the need to properly wash or cook vegetables prior to eating.

Materials and Methods

Study area and Sampling

The present study was carried out during the period from September, 2014, to August, 2015. The study included 590 vegetable samples, comprised of eleven types of vegetables and fruits: lettuce (80), watercress (116), parsley (88), green onion (100), onion (without leaves) (18) and leek (100), pepper (30), cabbages (10), carrot (20), fruits of tomato (12). The samples collected from farms, supermarkets, small markets and open aired markets. The open-aired markets were selected because they are noted for supplying not just to consumers but retailers as well. The samples were collected in two rounds of purchases, two weeks apart. Cleanliness of the samples was ranked as unwashed (presence of dust or other particulate matter), washed (no visible form of dust) and washed and packaged.

Sample Preparation

Samples were transported to the laboratory in sterile plastic bags. They were divided into three groups and each group washed with tap water, saline (0.85%) and phosphate buffered saline (PBS) (15). About 150 g of material was used for each vegetable except cabbage where one composite weighing about 400 g was used for each wash. Lettuces were separated into individual leaves whereas cabbages were divided in four. The samples were agitated vigorously (manually) in the respective liquids for about three minutes. The eluent was filtered through gauze and then dispensed into clean centrifuge tubes and centrifuged at 2000·g for 30 min. The supernatant was decanted and the residue was agitated gently in sucrose flotation medium of 1.18 specific gravity. Each tube was then topped to the brim with the flotation medium to form a meniscus. A

cover slip was placed on the test tubes for about 3 mins and this was removed and placed on a clean glass slide and viewed under microscope for the presence of parasitic oocysts. Oocysts positive slide was allowed to air dry and stained using modified Ziehl-Neelsen technique (WHO, 1990; Brondson, 1984) by fixing the air-dried slides in methanol for 2-3 minutes. The slide was flooded with cold carbolfuchsin for 5-10 minutes and then with 1% Hydrochloric-acid ethanol until color ceases to flow out and rinsed in tap water. Then the slide was counter stained with 0.25% methyl blue for 30 seconds, and rinse in tap water again and air dried.

Identification of *Cryptosporidium* oocysts

The slide was examined using a light microscope at 40 magnifications with oil immersion. The cryptosporidial oocysts appeared as bright rose pink spherules on a pale green background. Each oocysts present in each sample were enumerated were expressed as “many” (>three oocysts per high-power field; per low-power field); “moderate” (two oocysts/ high power field/ low power field); “few” (one oocyst/field).

Data Analysis

With the use of EPI INFO 2002 (Dean, 1994), Chi square test was used to test for association between *Cryptosporidium* oocysts and factors such as type, nature, consumption and source of vegetables. OR values greater than unity denote association and less than unity denote that the factor may have a protective effect. $p < 0.05$ was defined as significant. Prevalence was estimated by dividing the number of positive samples by the total number of samples.

Ethical approval was obtained from Ethics Committee of the University

Results and Discussion

Of the 590 vegetables examined, 107 (18.1%) were positive for *Cryptosporidium* oocysts. Ten varieties of vegetables were examined and all were positive for *Cryptosporidium* oocysts. Lettuce had the highest (26.3%) contamination rate followed by Leek (22%). There was no statistical significance ($\chi^2 = 8.70$; $p = 0.2747$) between occurrence of *Cryptosporidium* oocysts and types of vegetables examined (Table 1).

Table 2 shows the occurrence of *Cryptosporidium* oocysts in the different markets which were 224 (37.9%) of the vegetables examined from open aired market was positive for *Cryptosporidium* oocysts followed by farms 165 (27.9%) small market 130 (22%), and 71 (12%) from

supermarkets. There was no statistical significance ($\chi^2 = 0.22$; $p = 0.8959$) between occurrence of *Cryptosporidium* oocysts and the markets in which the vegetables were sold.

Contamination was highest in leafy vegetables (37.4%), followed by fruit vegetables (35.5%) and root vegetables (27.1%). There was no statistical significance ($\chi^2 = 1.79$; $p = 0.4091$) between occurrence of *Cryptosporidium* oocysts and the nature of vegetables examined (table 4).

The present study aimed at investigating some green vegetables that are frequently eaten raw for their possible contamination with *Cryptosporidium* oocysts in Cairo.

Table.1 Occurrence of *Cryptosporidium* oocysts and Types of Vegetables Examined

Sample (n= 590)	No. examined	No. positive	%
Lettuce	80	21	26.3
Leek	100	22	22.0
Parsley	88	18	20.5
Green Onion	100	19	19.0
Cabbage	10	1	10.0
Watercress	116	18	15.5
Onion	18	0	0.0
Carrot	20	4	20
Pepper	30	2	5.0
Tomato	12	2	16.7

P>0.05

Table.2 Prevalence of Vegetables at Different Markets. *Cryptosporidium* oocysts Contamination of Examined Fresh

Market n= 590	Contamination	
	No.	%
Farms	165	27.9
Supermarkets	71	12.0
Small markets	130	22.0
Open aired markets	224	37.9

Table.3 Seasonal Prevalence of *Cryptosporidium Oocysts* in Row Vegetable Plants

Year season	No examined	Positive	%
Summer	158	29	26.8
Autumn	122	22	17.3
Winter	108	18	16.6
Spring		38	35.1
Total	590	107	18.1

p<0.05 significant difference between different seasons

Table.4 Percentage of Contamination Sample with *Cryptosporidium oocysts* in Different Season in Examined Fresh Vegetables

Sample	Summer		Autumn		Winter		Spring		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Leek	6		3		1		11		21	19.6
Lettuce	7		4		1		10		22	20.5
Parsley	4		4		3		3		14	13.1
Green Onion	4		3		3		9		19	17.8
Cabbage	1		0		1		0		2	1.9
Watercress	3		6		5		0		14	13.1
Carrot	2		2		2		1		7	6.5
Pepper	1		0		2		2		5	4.7
Tomato	1		0		0		2		3	2.8
Total	29		22		18		38		107	100

Table.5 Occurrence of *Cryptosporidium Oocysts* and the Nature of Vegetables Examined

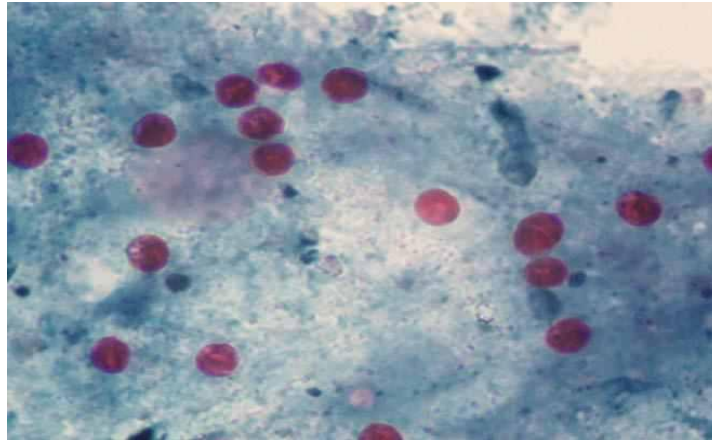
Type	No	%
Leafy vegetables	40	37.4
Fruit vegetables	38	35.5
Root vegetables	29	27.1
	107	100

Figure.1 Sample Collection Sites and Forms of Display



Fresh vegetables from different market (supermarket, small market and open air market)

Fig.1 Modified Ziehl-Neelsen Stained Smear Showing *Cryptosporidium* oocysts



Responses from the retailers show that about 30% of the vegetables were gotten straight from farm while 70% were from middlemen who got them from farms and sold to retailers in the market before being sold to consumers. Likewise, vegetables were either washed in the farms and when brought to the market water from tap are used to constantly sprinkle on them to prevent from drying or they are washed in the market when brought in using tap water.

The 18.1% contamination rate of *Cryptosporidium* oocysts in this study is relatively high compared to the work of others (Monge *et al.*, 1996). This may be due to the use of dirty water in washing the vegetables because as a routine, traders usually sprinkle vegetables with water to preventing them from drying. Water has been recognized as an important vehicle for the transmission of *Cryptosporidium* (Rose *et al.*, 1990). The ubiquitous nature of this protozoan parasite and its potential for waterborne transmission is further facilitated by the perpetual infectivity of the oocysts, their small size (3.5e6.0 mm) and their low sedimentation rate (0.5 mm/s)(20). Surface water may become contaminated through the entry of human or animal faeces by either a direct or indirect route. Direct contamination of water may be due to the

entry of faeces from agricultural run-off from adjacent farm animals or indirectly through accidental contamination from human sewage (Peng *et al.*, 1997). Contact of the vegetables with soil may also play significant role in the contamination of the vegetables with *Cryptosporidium* oocysts as the vegetables were kept in dirty environment and in contact with soil. Also fertilization of horticultural crops, including vegetables, with manure from cattle and sheep, containing viable oocysts of *Cryptosporidium*, represents a significant risk to the contamination of vegetables (Moore *et al.*, 2007).

The high occurrence of *Cryptosporidium* oocysts in lettuce maybe probably due to its broad leafy nature which may enhance it trapping higher number of oocysts from contaminated sources. Contamination of lettuce with food poisoning and foodborne pathogens may occur in the fields as a result of the introduction of the organisms from non-potable irrigation water or manure/compost, which still contains viable pathogens. There has been a number of lettuce associated food borne pathogens and the consumption of raw vegetables plays an important role in the transmission of parasitic contaminations (Anuar *et al.*, 1977). A recent paper by researchers in USA

demonstrated that *Cryptosporidium* oocysts were capable of strongly adhering to spinach plants after contact with contaminated water and in a variety of ways, it is mainly associated with the water used for irrigation. The use of sewage contaminated water for irrigation of vegetables is a common. The highest rate of contamination was detected in Green leafy vegetables (37.4%). This could be due to the fact that the degree of contamination varies according to the shape and surface of vegetables. Green leafy vegetables as lettuce, have uneven surfaces and makes *Cryptosporidium* oocysts attached to the surface of the vegetable more easily, either in the farm or when washed with contaminated water. On the other hand, vegetables with smooth surface as leek and green onion had the least prevalence because its smooth surface reduces the rate of oocysts attachment. Some other studies have also demonstrated a higher contamination rate in leafy vegetables.

In conclusion, since vegetables are an important part of a healthy diet and can be consumed either raw or cooked, the findings from this research study is of great public health significance. The findings may have important implications for global food safety and emphasize the importance of raw vegetables in threatening public health by transmission of *Cryptosporidium* oocysts to humans in Egypt. The local health and environmental authorities should improve the sanitary conditions in the areas where the vegetables are cultivated and consumed. Proper treatment of wastewater used for irrigation of vegetables should be implemented. There is also dire need for the improvement of sanitary facilities in our markets and vegetable vendors. Media programs should inform the consumers the potential health consequences of the consumption of raw vegetables, and the importance of proper washing and

disinfecting of vegetables before consumption. In addition, they should focus on the necessity of good sanitation hygiene and risks of acquiring infection. More researches are needed to do surveys of contamination in green vegetables indifferent districts. Also, other surveys must be done in different governorates in Egypt. Also, other researches must be done to evaluate the level of contamination of irrigation water and soil in which green vegetables are cultivated. Different ways of disinfection of raw green vegetables must be improved.

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How to cite this article:

Gehad T. El Sherbini, Nancy Osman Hany Kamel, Morsy R. Geneedy and Ashraf G. Temsah. 2016. A Comparative Study of the Occurrence of *Cryptosporidium parvum* oocysts found on Fresh Fruits and Vegetables Sold in Supermarkets and Open-aired Markets. *Int.J.Curr.Microbiol.App.Sci.* 5(8): 760-768. doi: <http://dx.doi.org/10.20546/ijcmas.2016.508.085>