

Detection of *Cryptosporidium oocysts* in Commonly Consumed Fresh Salad Vegetables

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Abstract The aim of this study was to determine the degree of contamination caused by *Cryptosporidium oocysts* in regularly consumed salad vegetables sold at various wholesale and retail markets in northern part of Bangladesh. A total number of 165 samples of salad vegetables collected from different wholesale and retail markets were examined for detection of *Cryptosporidium oocysts* using sucrose flotation medium of 1.18 specific gravity and Ziehl Neelsen staining technique with some modifications. *Cryptosporidium oocysts* were detected in 47 (30%) of the total examined samples. About 40 Tomato, 35 Cucumber, 20 Lettuce, 35 Carrot and 35 Mint's leaf samples were examined while Lettuce had the highest (40%) contamination rate followed by Tomato (32.5%), Carrot (31.4%), Cucumber (25.7%), and Mint's leaf (22.8%). There was no significant difference ($x^2 = 2.278$; $p < 0.05$) among occurrences of *Cryptosporidium oocysts* in usually consumed salad vegetables sold at market. This study has shown that salad vegetables sold at wholesale and retail markets in northern part of Bangladesh are contaminated with *Cryptosporidium oocysts*, may pose a health risk to consumers of such products. This reveals food safety and significance of public health.

Keywords: salad vegetables, *Cryptosporidium oocysts*, food safety, northern Bangladesh

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1. Introduction

Salad vegetables are most popular worldwide due to their nutritional value. These vegetables are rich sources of beneficial anti-oxidants, minerals, vitamins and fibers [1]. Regular consumption of green salad vegetables may reduce the risk of cardiovascular diseases, stroke and certain cancers [2]. But eating of salad vegetables may lead to ingest intestinal parasites which may causes digestive disturbances in human body such as diarrhoea, dysentery, bloating and flatulence, obstruction, fatigue and anemia [3]. The main route of transmission of parasites diseases is the eating of raw vegetables without proper washing [4]. Outbreaks of intestinal parasitic infections have been reported from developed and poor countries linked to raw vegetables [5,6]. Raw vegetables may be contaminated with parasitic pathogens such as *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 [7,8,9].

Cryptosporidium is a most common parasite and can contaminate salad vegetables if these vegetables are irrigated with contaminated water which is defaecated by both human and animal due to poor hygienic condition of the environment [10]. *Cryptosporidiosis* is a most common parasitical infection caused by *Cryptosporidium oocysts* and microbial cause of diarrhea along with noticeable weight loss, pain and abdominal colic [11] *Cryptosporidiosis* can become severe and not solving problem in children and also in case of immune-compromised individuals. It may remain for up to five weeks in the lower intestine of the human body [12]. *Cryptosporidium* infection is transmitted by ingesting of *Cryptosporidium oocysts* through feacally contaminated water or food [13].

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

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

2. Materials and Methods

2.1. Sample Collection and *Cryptosporidium oocysts* Analysis

A total number of 165 samples of the following five types of vegetables were collected from different wholesale and retail markets of the northern part of Bangladesh.

English name	Botanical name	Nature of vegetables
Tomato	<i>Lycopersicon esculentum</i>	Fruit
Lettuce	<i>Lactucasatva</i>	Leafy
Carrot	<i>Daucuscaritavar sativa</i>	Root
Cucumber	<i>Cucumis sativus</i>	Fruit
Mint's leaf	<i>Menthaspicata</i>	Leafy

The samples were collected into clean polythene bags throughout the year. They were transported to laboratory of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The methods of [3,11] with some modifications were used for detection of *Cryptosporidium oocysts* in salad vegetables as follows: 250 g of each sample was soaked in 150 ml of the physiological saline solution (0.98% NaCl) in 1000 ml beaker. With the aid of mechanical shaker, the beaker was shaken at 150 rpm for 30 minutes. After that, the wash was left for 45 minutes for sedimentation to take place. The supernatant was discarded and residue was transferred into a centrifuge tube and spun for 5 minutes at 1500 rpm. 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 [14,15] by fixing the air-dried slides in methanol for 2-3 minutes. The slide was flooded with cold carbol fuchsin 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.

2.2. Identification of *Cryptosporidium oocysts*

The slide examined using a light microscope at 40 magnifications with oil immersion. The *Cryptosporidium oocysts* appeared as bright rose pink spherules on pale green background.

2.3. Data Analysis

Using Microsoft Excel 2007, Chi square (χ^2) test was done and this test was used to test for association between *Cryptosporidium oocysts* and types of salad vegetables from different markets. Occurrence was determined by dividing the positive number samples by the total number of samples.

3. Results and Discussion

A Total number of 165 salad vegetable samples examined, 49 (30%) of which were positive with *Cryptosporidium oocysts*. In this study five varieties of salad vegetable were examined and all were positive with *Cryptosporidium oocysts* contamination. Among of all examined samples lettuce had showed the highest (40%) contamination rate followed by tomato (32.5%), carrot (31.4%), cucumber (25.7%), and mint's leaf (22.8%). There was no significant difference ($\chi^2 = 2.287$; $p < 0.05$) among occurrences of *Cryptosporidium oocysts* in commonly used salad vegetables sold at wholesale and retail markets (Table 1).

Table 1. Contamination of green salad vegetables with *Cryptosporidium oocysts* in markets of the northern part of Bangladesh

Vegetables		No. of vegetables Examined	Positive samples		Chi square (χ^2)	Degree of freedom	p- value
English name	Botanical name		No.	%			
Tomato	<i>Lycopersicon esculentum</i>	40	13	32.5	2.287	4	0.05
Lettuce	<i>Lactuca satva</i>	20	8	40			
Carrot	<i>Daucuscaritavar sativa</i>	35	11	31.4			
Cucumber	<i>Cucumis sativus</i>	35	9	25.7			
Mint's leaf	<i>Mentha spicata</i>	35	8	22.8			
Total		165	49	30			

In this study, the rate of contamination of green salad vegetables with *Cryptosporidium oocysts* is 30% which is slightly lower compared to the work of others [16] (Figure 1). This result may be owing to use of dirty water in washing vegetables because as a routine, traders sprinkle vegetables with water to preventing them from drying after harvesting and during selling them in a market. As a main route for transmission of *Cryptosporidium* water has been recognized [17].

In this research study, five major types of raw salad vegetables from different markets of northern part of Bangladesh were examined; lettuce, tomato, carrot, cucumber and mint's leaf. The maximum contamination

rate was detected in lettuce samples (40%) while mint' leaf sample had lowest contaminated with *Cryptosporidium oocysts* (Figure 2). This may possibly be because of the fact that the degree of contamination varies according to shape and surface of vegetables [1]. Salad vegetables as lettuce, has bumpy surfaces and makes parasitic oocysts attached to the surface of the vegetable more easily, during harvesting in the farm or when washed with dirty water to preventing them from drying. On the contrary, vegetables with flat surface as cucumber, tomato and carrot had the least occurrence as its flat surface reduces the rate of parasitic attachment [18]. These results were similar with that of a study made in Tripoli, Libya

[19]. According to this study *Cryptosporidium oocysts* contamination was 96% in lettuce sample. Lettuce was contaminated significantly more often than those of others samples in the same study. According to [18], contamination rate was detected 40% of lettuce samples and 24% in other raw vegetable in Nigeria. The rate of contamination can vary depending upon the Geographical location, type of samples, methods used for detection, type of water source used for irrigation and post-harvest processing of such vegetables which are different from country to another.

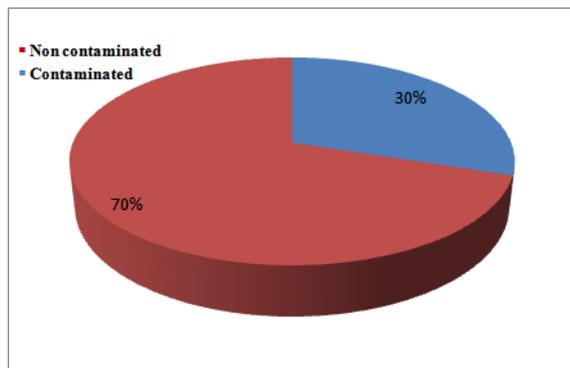


Figure 1. Percentage of the total number of contaminated and non contaminated with *Cryptosporidium oocysts* samples of raw salad vegetables at different markets

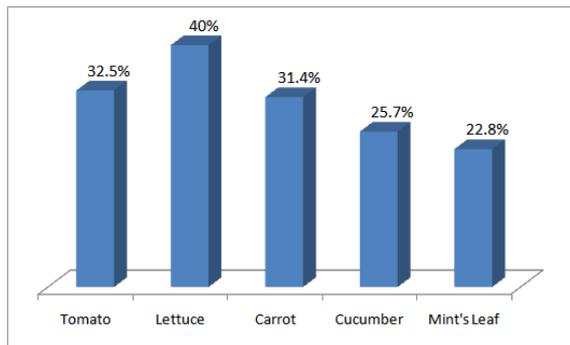


Figure 2. The percentage of contamination of each type of salad vegetables with *Cryptosporidium oocysts* at different markets

4. Conclusion

Contamination of green salad vegetables with *Cryptosporidium oocysts* sold in wholesale and retail markets in Bangladesh may cause a health risk to consumers of such products. The environmental and local health authorities of government should train the people about health risks of raw salad vegetables and the significance of proper pretreatments such as washing and disinfecting them prior to eating. These authorities should improve the sanitary conditions in the areas where the vegetables are cultivated and harvested and also dire need for the development of sanitary facilities in our markets and vegetable vendors. Since green salad vegetables are consumed as raw and also essential part of a healthy diet, the findings from this research study reveal food safety and health hazards of eating raw salad vegetables and also initiatives should be taken to prior to consumption of fresh vegetables and great public health significance.

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