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Investigation of *Cryptosporidium* oocyst in vegetables in Bangladesh

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ABSTRACT

The protozoa under the genus *cryptosporidium* are zoonotic apicomplexan obligate intracellular parasites which infect the intestinal epithelium of diverse mammals including humans, and cause a diarrheal disease, cryptosporidiosis. Cryptosporidiosis is a zoonotic disease and a major threat to human health due to the omnipresent distribution of *Cryptosporidium* species affecting humans and animals and due to the resistance of the oocysts to harsh environmental conditions and various disinfectants. Vegetable act as a great reservoir for contamination and a potential transport medium of the pathogens. The study was aimed to isolate and identify *Cryptosporidium* oocysts in vegetable samples in Bangladesh. Among various types of vegetables, red spinach, fresh coriander, water spinach and jute leaf samples were collected from different areas of Mymensingh and Sherpur districts of Bangladesh. To detect *Cryptosporidium*, the samples were assessed by microscopy, using the conventional Ziehl-Neelsen staining method. Overall 10 (16%) of 63 samples examined by microscope, were tentatively found as positive for cryptosporidium oocysts. Altogether, 6 (15%) of 40 samples, examined by microscopy, were tentatively detected as positive for *Cryptosporidium* oocysts in Mymensingh district. In Sherpur district, 4 (17%) of the 23 samples examined by microcopy were found as positive. The results represent the presence of *Cryptosporidium* in different types of sources of vegetables in both Mymensingh and Sherpur districts. However, further studies are needed to explore the molecular characters at genotype and subtype level for better understanding the transmission dynamics of the parasite.

Keywords: *Cryptosporidium*, oocyst, vegetables, Bangladesh.

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INTRODUCTION

The protozoa under the genus *Cryptosporidium* is a zoonotic apicomplexan obligate intracellular parasite (Rossle and Latif, 2013). Cryptosporidiosis, the term used to designate infection caused by *cryptosporidium spp*. is considered as one of the most common food and water borne disease with worldwide spread, acting as a common cause of diarrhea in animals and man. The infection is usually self-limiting in immune-competent individuals, but fatal in immuno-compromised individuals, e.g., acquired immune deficiency syndrome (AIDS) or leukaemia patients, taking immunosuppressive agents, malnourished children and elderly individuals (Current et al., 1983; Alves et al., 2001; Mohandas et.al., 2002). Cryptosporidiosis is prevalent worldwide (Dalle et al., 2003; Leoni et al., 2007; Lake et al., 2008; Zintl et al., 2008) including Bangladesh (Rahman et al., 1985). *Cryptosporidium* species are reported to be a significant cause of diarrhoeal illness of young children especially less than 5 years of age in Bangladesh (Rahman et al., 1990; Bhattacharya et al., 1997, Albert et al., 1999). About a decade ago, infection with *Cryptosporidium* species were reported in 1.4 -8.4% diarrhoeal patients (Haque et al., 2003; Khan et al., 2004) from International

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Centre for Diarrheal Disease Research, Bangladesh in Dhaka, (ICDDR'B).

Vegetable is the great reservoir for contamination and potential medium of transmission of the parasite. In our country most of the farmers are not aware about proper management of waste, products in the livestock yard. Due to improper and poor hygienic management *Cryptosporidium* oocysts from animal waste in barnyards, manure pits and field application can contaminate vegetables. In rural and semi-urban areas farmers wash their harvested vegetables in pond, lake, or river water where people usually take their bath, wash their cloths, and take the water to their house for drinking and household uses. Most of the slums have hanging latrines on the water bodies which is making the water bodies polluted and this polluted water frequently used in cultivation of vegetables. Stools from the children or adults outside the sanitary toilet directly used as manure. Dog, cat and other animals also defecate in the cultivable vegetable land that directly contaminates vegetables.

Cryptosporidium oocysts, which are excreted by infected animals and humans, are rarely found in different types of vegetables especially in those areas where lakes, rivers and ponds water are used. There are some other factors responsible for the presence of *Cryptosporidium* oocysts in vegetables and these includes the use of cowdung and human excreta in cultivable lands. The pilling of manure here and there is also a factor associated with this because in rainy season these manure are washed away through rain water. *Cryptosporidium* oocysts are resistant to normal environmental condition and common disinfectants. Traditional epidemiological investigation only provides information on the prevalence of this parasite but does not provide any information for tracking infection sources and or transmission dynamics of cryptosporidiosis. Molecular epidemiologic studies using genotyping and sub typing tools have led to better appreciation of the public health importance of *Cryptosporidium* species/genotypes in various animals and improved understanding of infection sources in humans. Geographic, seasonal and socioeconomic differences in the distribution of *Cryptosporidium* in human have been identified, and have been attributed to differences in sources of infection and routes of transmission. Molecular epidemiological studies have significantly improved our knowledge of cryptosporidiosis. Recently, molecular diagnostic tools have been developed to assess the human infection potential of *Cryptosporidium* oocyst in vegetables and to track the sources of contamination. Therefore, the study was conducted to isolate and indentify *Cryptosporidium* oocysts from different sources of vegetables in Sherpur and Mymensingh district using conventional technique to have clear and solid epidemiological information of the pathogen.

MATERIALS AND METHODS

Study area and period

Vegetable samples were collected from Mymensingh and Sherpur district of Bangladesh. In Mymensingh district samples were collected from Bangladesh Agricultural University Campus Area, Churkhai Bazar, Shesmore and Sutiakhali area. In Sherpur district, samples were collected from Nakla, Nalitabari and Sherpur Sadar. The vegetable samples were initially processed at the collection sites and then brought to the laboratory in the Department of Parasitology, Bangladesh Agricultural University, Mymensingh. Part of the work was done at the Laboratory of Department of Microbiology & Hygiene, Bangladesh Agricultural University Mymensingh. The study was conducted during the period of January 2015 to June 2015

Sample collection and processing

During the present study, a total of 63 vegetable samples of different varieties were collected from several markets and directly from the field where farmers use cowdung as bio-fertilizer. Almost one kilogram of each vegetable sample was collected in polythene bag and carried it to the laboratory. The samples were washed in big plastic jar vigorously and than initially passed through a mesh sieve (40mm mesh sieve) to remove coarse particle and after that 1 micron polyester filter bag (Duda, LLC, USA). The concentrated sample retained in the 1 micron filter bag was collected after mixing with 1% Teen-20 in a 50 ml tube. The samples were then centrifuged at 1500 rpm for 30

min for 2 times with distilled water for washing and again concentrated by flotation techniques using saturated salts solution. A drop of concentrate was smeared on slide and stained with modified Ziehl-Neelsen technique (Henriksen SA and Pohlenz, 1981) and examined under microscope.

RESULTS AND DISCUSSION

Microscopic examination

Air dried slides were examined under light microscopy at X100 magnifications. Oocysts of *Cryptosporidium* were found as pink colored round and spherical body as they took carbol fucshin stain. The back ground of the slide took blue color of methylene blue (Figure 1).

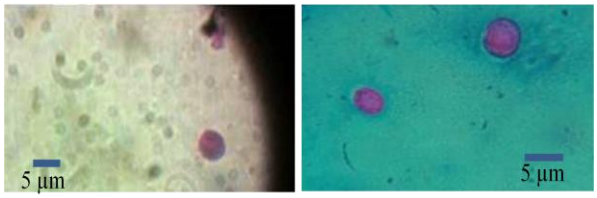

Figure 1
Oocysts under microscope (100X). Pick colored round or oval shaped oocyst of Cryptosporidium with (left) or without (right) blue background.

Red spinach, fresh coriander, jute leaf and water spinach samples were selected for the identification of oocyst of *Cryptosporidium* sp. A total of 63 vegetable samples were collected from two districts such as Mymensingh and Sherpur. Of them, 40 samples were collected from Mymensingh district and 23 samples were collected from Sherpur.

Occurrence of *Cryptosporidium* in Mymensingh and Sherpur districts

In Mymensingh district, *Cryptosporidium* was found 20% in red spinach sample, 30% water spinach samples and 10% in jute leaf sample whereas no oocysts was detected in fresh coriander samples (Table 1).

Table 1
Identification of *Cryptosporidium* by Ziehl Neelsen stain from samples collected from Mymensingh.

Types of Samples	Number of samples	No of positive samples	Percent positive samples
Red spinach sample	10	2	20%
Fresh coriander Sample	10	0	0%
Water spinach sample	10	3	30%
Jute leaf Sample	10	1	10%
Total	40	6	15%

Samples were considered positive by observing the presence of at least 2 oocysts of *Cryptosporidium*

Table 2
Detection of *Cryptosporidium* by Ziehl Neelsen stain from samples of Sherpur.

Name of Samples	Number of samples	No of positive samples	Percent positive samples
Red Spinach sample	5	1	20%
Fresh Coriander Sample	5	0	0%
Water Spinach sample	8	2	25%
Jute Leaf Sample	5	1	20%
Total	23	4	17%

In Sherpur district, 25% water spinach samples, 20% red spinach samples and 20% jute leaf samples were *Cryptosporidium* positive whereas no fresh coriander samples were positive for *Cryptosporidium* (Table 2).

Oocyst of *cryptosporidium* was detected commonly by using Ziehl-Neelsen technique during this study. Conventional methods include examination of fecal smears with acid-fast stains such as Ziehl-Neelsen (Scott, 1988), which is commonly used by diagnostic facilities. Conventional microscopy, however, is time-consuming and tedious and requires experienced person to accurately identify the oocysts (Garcia *et al.*, 1987; Kehl *et al.*, 1995) but still in use in many laboratories of the world as a cost-effective detection method of *Cryptosporidium*. In this study *Cryptosporidium* oocyst from concentrated vegetable sample was examined under microscope following stained with Ziehl-Neelsen stain for screening of the sample.

Altogether, 6 (15%) of 40 samples, were tentatively detected as positive for *Cryptosporidium* oocysts in Mymensingh district. In Sherpur district, 4 (17%) of the 23 samples were found as positive for *cryptosporidium* oocysts. The overall prevalence of *Cryptosporidium* in vegetable samples was 16% in this study which is in conformity with the study of Duedu et al. (2014). In Mymensingh district the highest prevalence was recorded in water spinach (25-30%) because naturally it grows alongside water and water is a great reservoir of *Cryotosporidium*. Similar findings were observed by Maikai et al. (2013) who reported higher prevalence of *Cryptosporidium* in spinach and Jute mallow (40%). Feces from *Cryptosporidium* infected wild or firm animals may directly contaminate vegetable.

The presence of *Cryptosporidium* oocysts in vegetable samples from the sampling sites confirmed the presence of *Cryptosporidium* species in different vegetable in Mymensingh and sherpur districts of Bangladesh. The study also indicates that *Cryptosporidium* might come from animal or human. Since *Cryptosporidium* is a zoonotic disease, there is risk of infection with *Cryptosporidium* in people and animals living in these areas.

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