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# Isolation and identification of *Cryptosporidium* from fecal sample of calves in Mymensingh and Kurigram district

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ABSTRACT

Cryptosporidiosis, a neglected parasitic disease, presents a serious threat to human health due to the ubiquitous distribution of *Cryptosporidium* species affecting humans and animals, and due to the resistance of the oocysts to harsh environmental conditions, various disinfectants, and some common therapeutic agents. Feces is a great reservoir for contamination and a potential medium of transmission of the pathogen. Here, study was aimed to isolate and identify *Cryptosporidium* from fecal samples in Bangladesh. Calves feces samples were collected from Mymensingh and Kurigram district of Bangladesh. In Mymensingh district samples were collected from Bangladesh Agricultural University campus area, Churkhai and Sutiakhali area. In Kurigram district samples were collected from Chilmari, Ulipur & Dahobandh area. *Cryptosporidium* was detected by the conventional Ziehl Neelsen staining method. By microscopy, *Cryptosporidium* oocysts were detected. The results indicate the existence or prevalence of *Cryptosporidium* in the study areas. In Mymensingh district, *Cryptosporidium* was found in 25% samples of which 30% calf were at the age of 1 to 15 days and 40% were at the age of 15 to 30 days & 0% at the age of 30 to 180 days. *Cryptosporidium* was positive in 33% fecal samples collected from Kurigram distric of which 33% fecal from the age of 1 to 15 days and 40% were at the age of 15 to 30 days & 30% at the age of 30 to 180 days calf. However, further studies are need to explore the present status of prevalence of *Cryptosporidium* in humans and animals in order to better understanding the transmission dynamics of the parasite and thereafter taking necessary measure to control and/or prevent the disease.

## Introduction

The genus *Cryptosporidium* includes a group of intracellular protozoan parasites that infect the gastrointestinal tract and other organs of mammals including human, birds, reptiles and fishes (Xiao et al., 1999). *Cryptosporidium* is an important cause of diarrheal illness and its impact increasing both local and globally. Widespread outbreaks of disease initiated public and animal health problems both in developed and developing countries (Fayer & Xiao, 2007).

*Cryptosporidium* is a zoonotic protozoan parasite which infects the intestinal epithelium of diverse mammals including humans, and causes a diarrheal disease, *Cryptosporidiosis* (Tzipori & Ward, 2002). The infection is usually self-limiting in immune-competent individuals, but fatal in immune compromised individuals, e.g. people suffering from acquired immune deficiency syndrome (AIDS), leukaemia or taking immunosuppressive agents, malnourished children and elderly individuals (Alves et al. 2001; Mohandas, 2002). Cattle are major hosts of *Cryptosporidium spp. Cryptosporidiosis* in neonatal calves is associated with retarded growth, weight loss and calf mortality, and zoonotic infections in humans. In many areas, cow-calf grazing system is important for beef cattle rearing method with distinct advantages in terms of cost and the labor required (Dalle et al. 2003; Leoni et al. 2007; Lake et al. 2008).

*Cryptosporidium* species have been reported to be a significant cause of diarrhoeal illness in 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 about 1.4 -8.4% of diarrhoeal patients (Haque et al. 2003; Khan et al. 2004) from International Centre for Diarrheal Disease Research, Bangladesh in Dhaka, (ICDDR'B). On the other hand, about three decades ago, the disease was also found prevalent among the animal population in Bangladesh (Rahman et al. 1985). Clinical cases in most of places in Bangladesh are treated as undiagnosed diarrhoeal patients. Despite enormous effect on animal and human health, presently very little attention has been paid to determine the present status of the infection calves in Bangladesh.

*Cryptosporidium* oocysts, which are excreted by infected animals and humans, are commonly found in feces of infected animal. Feces are the most important source for contamination. Therefore, the present study was conducted to determine

*Cryptosporidium* infection in calves in Kurigram and Mymensingh districts of Bangladesh.

## Materials and Method

### Study area and period

Calve feces were collected from Mymensingh and Kurigram districts of Bangladesh. In Mymensingh district, samples were collected from Bangladesh Agricultural University campus area, Churkhai and Sutiakhali area. In Kurigram district, samples were collected from Chilmari, Ulipur & Dahobandh area. The fecal samples were initially processed at the collection sites and, then brought to the laboratory at the Department of Parasitology, Bangladesh Agricultural University, Mymensingh. The study was conducted during the period of January 2015 to May 2015.

### Sample collection and processing

A total of 50 samples from calves were collected from Mymensingh and Kurigram districts. Of them, 20 samples were collected from Mymensingh district and 30 samples were collected from Kurigram. The fresh faecal samples were collected in a plastic container with a detailed history about age group, breed and sex and then labeled with particulars of an individual animal on the container. Each sample was studied macroscopically to establish its consistency as liquid, soft or solid, and the presence of mucus or blood was noted. In laboratory the suspected sample was mixed with tap water with sufficient volume and passed through a mesh sieve (40mm mesh sieve) to remove course particle. The samples were then centrifuged at 1000 rpm for 10 min for 2 times with distilled water for washing. The sample was then concentrated by flotation techniques using saturated salts solution. A drop of concentrate was smeared on slide and stained with modified Ziehl-Neelsen technique (Henriksen & Pohlenz, 1981) and examined under microscope (Figure 1). The positive samples were washed with distilled water and stored at 4°C.

### Microscopic examination

Air dried slides were examined under light microscopy at ×40 and ×100 magnifications. Oocysts of *Cryptosporidium* were found as pink colored round and spherical body as it takes carbol fuchin stain. The back ground of the slide takes blue color of methylene blue.

## Results and Discussion

A total of 50 samples from calves were collected from two districts such as Mymensingh and Kurigram. Of them, 20 samples were collected from Mymensingh district and 30 samples were collected from Kurigram.

### Detected by microscopy

Smear was prepared from the concentrated samples and stained with Ziehl Neelsen stain as done by Sharifuzzaman et al. (2015). Oval or round shaped pink color oocysts of *Cryptosporidum* were observed under microscope with blue background. In Mymensingh district, prevalence of *Cryptosporidium* infection was 25%, however 33% sample were ware positive from all sample collected from Kurigram district.

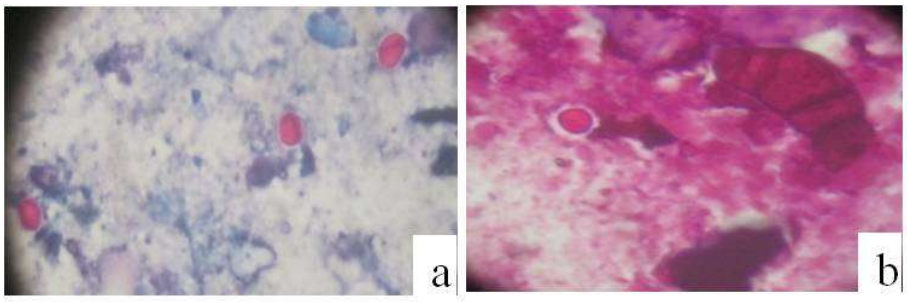


Figure 2. Oocyst under microscope (×100) (a) Pink colored *Cryptosporidium* oocyst with blue background (destained). (b) Pink colored *Cryptosporidium* oocyst without blue background.

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

| Age of animal (days) | No. of sample | No. of positive sample | Prevalence |
|---|---|---|---|
| 1 to 15 | 10 | 3 | 30% |
| 15 to 30 | 5 | 2 | 40% |
| 30 to 180 | 5 | 0 | 0% |
| Total | 20 | 5 | 25% |

Samples were identified as positive by observing the presence of at least 2-3 oocysts of *Cryptosporidium* per focus of a slide.

### Effects of age of the animal

The study revealed that age of the animals had significant effects on the occurrences of the parasitic infection in Mymensingh district among the tasted animals, 30% calve at the age of 1 to 15 days and 40% were infected with the protozoa at the age of 15 to 30 days and importantly no

infection was detected at the age of 30 to 180 days (Table 1).

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

| Age of animal (days) | Total no. of sample | No. of positive sample | Prevalence |
|---|---|---|---|
| 1 to 15 | 15 | 5 | 33% |
| 15 to 30 | 5 | 2 | 40% |
| 30 to 180 | 10 | 3 | 30% |
| Total | 30 | 10 | 33% |

On the other hand in Kurigram district, 33% calves at the age of 1 to 15 days, 40% at the age of 15 to 30 days and 30% at the age of 30 to 180 days were infected with *Cryptosporidium* (Table 2).

During this study, oocyst of *Cryptosporidium* was detected by Ziehl-Neelsen technique. There are several methods for detection of *Cryptosporidium* under microscope. 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 is used in many laboratories of the world as a cost-effective detection method of *Cryptosporidium*. In this study *Cryptosporidium* oocyst from concentrated fecal sample was examined under microscope following stained with Ziehl-Neelsen staining. The findings of the study conform to those of Hassan et al. (2012) who found *Cryptosporidium* in 27.5% fecal sample by microscopy.

## Conclusions

Collectively, the results confirm the presence of *Cryptosporidium* in calves in the study areas. However further studies are needed to explore the present status of prevalence of *Cryptosporidium* in humans in order to better understanding the transmission dynamics of the parasite and thereafter, taking necessary measure to control and prevent of *Cryptosporidium* in Bangladesh.

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