PREVALENCE OF CRYPTOSPORIDIOSIS IN CROSSBRED CALVES IN TWO SELECTED AREAS OF BANGLADESH

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ABSTRACT

A cross-sectional study was conducted to determine the prevalence of bovine cryptosporidiosis using 110 fecal samples of crossbred diarrhoeic calves from two different areas (Muktagacha, Mymensingh and Shajadpur, Sirajgonj) in Bangladesh during April 2012 to September 2014. The fecal samples were screened by rapid detection kit and confirmed by Modified Ziehl-Neelsen staining, and polymerase chain reaction (PCR). The positive samples along with standard positive control yielded 1325bp band on PCR. The overall prevalence of cryptosporidiosis in crossbred calves was 28.18% (31/110) by rapid detection kit. The higher prevalence of cryptosporidiosis was found in the calves from Shajadpur (29.76%) than the calves from Muktagacha (23.08%). The prevalence of cryptosporidiosis was significantly (p<0.001) higher in calves between 1-2 months (70%) age group than less than one month age group (24.49%). Cryptosporidiosis was not observed in calves over two months age. The prevalence of cryptosporidiosis was higher in males (34.75%) than females (24.64%) although not significant statistically. It is evident that the prevalence of cryptosporidiosis in bovine in these areas is under diagnosed and the clinical status of infection is potentially high.

Key words: Cryptosporidiosis, Prevalence, Calves, Oocysts,

INTRODUCTION:

Cryptosporidiosis is an emerging zoonotic disease of global importance caused by the apicomplexan protozoan parasite which is one of the most common causes of diarrhea in humans and livestock worldwide. Oocysts of cryptosporidium are usually transmitted by the feco-oral route, through direct host-to-host contact, and indirect contamination of food or water (Sayers et al., 1996). The zoonotic transmission has been confirmed by epidemiological studies involving pets, farm animals and by accidental infection of veterinary workers (Ahmed., 1984; Webster, 1993; Casemore et al., 1997; Saini et al., 2000; Nydam et al., 2005; Collick et al., 2006). Ruminants are reported to be the major source of Cryptosporidium (C.) parvum transmission to humans (Xiao et al., 2004a, b; Caccio, 2005). Cryptosporidium spp. infection is well known as a major cause of morbidity and mortality particularly in immune compromised hosts and young animals (Graff et al., 1999). It causes self-limited watery diarrhoea in immunocompetent subjects but has far more devastating effects on immunocompromised patients and in some cases can be life threatening due to dehydration caused by chronic diarrhea (Alves et al., 2001; Mohandas et al., 2002; Caccio et al., 2005; Chen et al., 2005). In livestock the disease may lead to economic loss due to mortality, retarded growth of the animals, cost of drugs, veterinary assistance and increased staff labor (De Graaf et al., 1999). It has been reported as an important cause of calf mortality (Moon et al., 1982; Tzipori et al., 1989). Cryptosporidium oocysts may remain viable in water for over 140 days and are very resistant to the most common disinfectants making them difficult to destroy by conventional chlorination treatment (Ahmed, 1984).

Cryptosporidiosis is considered as the third major cause of diarrheal disease worldwide (Janoff and Reller, 1987; Casemore et al., 1997; Fayer et al., 1997, 2000; Morgan et al., 1999; Spano and Crisanti, 2000). Cryptosporidiosis in cattle has been reported from different parts of the world with prevalence ranging from 24.5% to 45.5% (Kumar et al., 2005). The calf mortality in Bangladesh up to 12 months of age was reported from 9% under rural (Debnath et al., 1990) to 13.4% under a farm (Debnath et al., 1995) conditions. Reports on entero-pathogens associated with calf diarrhea are very limited from Bangladesh (Samad et al., 1977, 2001). There is no published report on the prevalence of cryptosporidiosis in crossbred calves in Bangladesh. This study describes the prevalence of cryptosporidiosis in crossbred calves under large and small holder dairy farms in some selected areas of Bangladesh

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MATERIALS AND METHODS

Study areas, period and population
Shahjadpur Upazilla of Siragonj District and Muktagacha Upazilla of Mymensingh District in Bangladesh, the most important dairy zone of Bangladesh were selected as study area. Five hundred (500) farms having at least two cross-bred dairy cattle were selected conveniently. Calves from day old to 1 year of age were included in this study. Active monitoring and surveillance system was used to collect sample from the selected farms over a period of 30 months from April 2012 to September 2014.

Faecal sample collection and examination
A total of 110 faecal samples from diarrhoeic calves of research area (Muktagacha and Shajadpur) were collected and examined for the presence of Cryptosporidium. Fecal samples were collected directly from the rectum of the animals or from the faecal mass immediately after defecation in stool pot with detail history of age group and sex and were immediately capped, labeled accordingly which included sample identification and site of collection. The collected samples were placed on ice in an insulated container in order to maintain low temperature of the samples. Feces were transported to the Laboratory of Department of Medicine, Bangladesh Agriculture University, Mymensingh and processed within 1–3 days of collection. The samples were examined in the field and in the laboratory soon after collection or after preservation by freezing at -20°C. In this study, stool samples collected from diarrheic calves were tested by rapid detection kit (Rainbow Calf scour 5, BioX Diagnostics, Belgium) to detect Cryptosporidium and other enteropathogens from diarrheal fecal samples as per manufacturer’s instruction(Fig.1). A total of 4 representative samples from 31 positive for cryptosporidiosis by BioK-306 were mixed together and a thin smear was prepared and stained using a modified Ziehl-Neelsen method for further confirmation of Cryptosporidium spp (Fig-2). Samples were treated with carbol-fuchsin solution for 3 minutes recommended by Lennette et al., 1985. The discoloration procedure was realized with discoloration solution (95% ethyl alcohol-50ml and 95% Acetone-50ml) for 15-20 seconds used instead of the ethyl alcohol-sulfuric acid 5% recommended by Henriksen and Polhenz (1981). These modifications promoted a better washing out of the excess of carbol fuchsin therefore increasing the dye efficiency. In such conditions, the visualization of protozoan oocysts on the slides examined became easier. Smears were washed with running water and counterstained with solution of 0.4% malachite green or methylene blue at 1% for 1 minute. After the final wash with water, slides were dried at room temperature and then examined using X40 and X100 magnification under microscope. The positive samples were stored at -20°C for DNA extraction.

DNA Extraction
DNA was extracted from concentrated mixture of 4 positive samples. Cryptosporidium oocysts were purified using a NaCl flotation procedure. Purified oocysts were washed three times in DDW/PBS in 50ml tube. After wash, the sediment was re-suspended with 45ml saturated salt solution. Five milliliter DDW was layered above the re-suspended samples. Samples were then centrifuged at 2300rpm for 30 minutes without break. Cryptosporidium oocysts were deposited in the upper layer and were collected and transferred to another tube by pipette. The oocysts were then subjected to 5-8 freeze-thaw cycles and DNA extraction was carried out using a Promega DNA extraction kit according to the manufacturer’s instructions. The DNA extracted from the concentrated oocyst was used for polymerase chain reaction (PCR). The amplified products obtained from PCR assay were visualized after running through agarose gel electrophoresis.

Cryptosporidium detection by PCR
Cryptosporidium oocysts were confirmed by polymerase chain reaction (PCR). Primary PCR was performed by primers SSU-F2: (5 TTCTAGAGCTAATACATGCG 3) and SSU-R2: (5’-CCCATTCCCTTCGAAACAGGA 3) (Xiao et al.,1999; Limor et al., 2002; Park et al., 2006; Schindler et al., 2005). The primary PCR mixtures contained 5μl of template, 2X PCR Master mix (Promega, USA)-12.5μl, 1μl of each primer (10 pmol/μl) and DDW-5.5μl in a 25μl reaction volume. Thermocycling parameters were 3 minutes at 94°C hot start (initial heat activation step), followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 55°C and 1 minute at 72°C, with a final extension of 7 minutes at 72°C (Xiao et al., 1999). The PCR product was loaded on 1.5% agarose gel, electrophoresis was done for 1 hour. The gel was stained with ethidium bromide and the products (1325bp) were visualized under a UV transilluminator.
Prevalence of Cryptosporidium spp in crossbred calves

Statistical analysis
The association of cryptosporidiosis with other variable like area, age and sex were assessed by Chi-square test. The Chi-square test and 95% confidence interval of prevalence were performed in R 3.1.0 (The R foundation for Statistical Computing).

RESULTS AND DISCUSSION
A total of 110 fecal samples from diarrhoeic calves were examined where 31 samples were positive for Cryptosporidium spp. by rapid detection kit (Biok-306). Cryptosporidium spp oocysts were observed in stained smear (Fig. 2). The positive samples along with positive control yielded 1325bp (Fig. 3) band on visualization which was supported by earlier report (Hassanain et al., 2011). The overall prevalence of cryptosporidiosis in calves below six months of age was 28.18% (Table 1).

Prevalence of Cryptosporidiosis was higher in Shajadpur (29.76%) in comparison to that in Muktagacha (23.08%). However, this difference was not significant statistically (Table 1). Based on the results of our study, it is evident that bovine cryptosporidiosis is endemic and locally widespread in Bangladesh. Some studies have shown that Cryptosporidium oocysts are able to survive for extended periods in faeces and environment, and very low dose of viable oocysts can cause an infection (Chako et al., 2010). The apparent variability of prevalence between geographical localities may reflect differences in the levels of calf management practices employed at farm level, housing-related factors (i.e., single housed calves, cleanliness of the calf sleeping places), calf-related factors at a time of sampling (diarrhoeic versus non-diarrhoeic), nature of the study (cross-sectional versus prospective longitudinal studies), and fecal screening technique used (EL-Shazly et al., 2002; Kaushik et al., 2008). The prevalence of cryptosporidiosis was significantly (p<0.001) higher in calves between 1-2 months of age (70%) in comparison to those of one month of age (24.49%). This finding is also supported by other authors (Swai and Schoonman, 2010; Maldonado-Camargo et al., 1998; Gow and Waldner, 2006; Paul et al., 2008). Several authors reported higher prevalence among calves less than 6 months of age (Ongerth and Etibbs, 1989; Shovamoni, 2005; Jayabal and Ray, 2005; Roy et al. 2006; Mehdizami, 2007). The prevalence of cryptosporidiosis did not vary significantly according to sex of calves as also reported by others (Rehman et al., 1985; Shovamoni, 2005). However, Nouri and Toroghi (1991) recorded a higher infection in male diarrheic calves than in female calves.

Table 1. Prevalence of cryptosporidiosis in crossbred calves

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tested</th>
<th>Positive</th>
<th>Prevalence (%)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muktagacha</td>
<td>26</td>
<td>6</td>
<td>23.08</td>
<td>8.97-43.65</td>
</tr>
<tr>
<td>Shajadpur</td>
<td>84</td>
<td>25</td>
<td>29.76</td>
<td>20.27-40.73</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upto 1 month</td>
<td>98</td>
<td>24</td>
<td>24.49</td>
<td>16.36-34.21</td>
</tr>
<tr>
<td>More than 1 to 2 months</td>
<td>10</td>
<td>7</td>
<td>70.0</td>
<td>34.75-93.32**</td>
</tr>
<tr>
<td>More than 2 months</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0-70.76*</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41</td>
<td>14</td>
<td>34.15</td>
<td>20.08-50.59</td>
</tr>
<tr>
<td>Female</td>
<td>69</td>
<td>17</td>
<td>24.64</td>
<td>15.05-36.49</td>
</tr>
<tr>
<td>Overall</td>
<td>110</td>
<td>31</td>
<td>28.18</td>
<td></td>
</tr>
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</table>

*97.5% confidence interval ** Significant at p<0.001
CONCLUSIONS

Results of this study indicate that the prevalence of cryptosporidiosis in crossbred calves in these areas is underdiagnosed and the clinical status of infection is potentially high. The prevalence of the Cryptosporidium species/genotypes appeared to be age related. Because calves less than 3 months of age are the predominant population infected with C. parvum (zoonotic species), any effort designed to control this infection must be directed primarily at this age group.

A further prospective study, capturing seasonal variations to elucidate the magnitude of the disease (mortalities and reduced production), is desirable. Moreover, studies to understand the dynamics of transmission cycles and the genetic diversity of Cryptosporidium spp. on the farms should be undertaken.

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REFERENCES

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