**CHAPTER 1**

**INTRODUCTION**

Cattle have been reared in Bangladesh since early time of human settlement in the country. While they stand first in number among the ruminant species, the population of cattle in Bangladesh currently includes about 25.075 million animals, which are dispersed throughout the country (Anonymous, 2007). The average number of cattle per household is 2.459 and they are mostly reared by landless, small and medium farmers. Cattle are used primarily for meat production, milk production and their skin, horn, bone, blood are are valuable by-product.

Diseases are the major constraints of cattle production in Bangladesh. The mortality of cattle and calves is also another major problem confronting cattle rearing in Bangladesh. Like other livestock, Cattle is also susceptible to various diseases or disease conditions causing debility and mortality, which lead to huge economic loss. In Bangladesh, the common diseases which affect the young or adult cattle causing death are parasitism, infectious diseases like FMD, BQ, HS, Anthrax etc.

In parasitic infection main symptoms is diarroea, there are many parasite causing diarrohea, protozoa is one of them. There is different type of protozoa like *Cryptosporidium* *Spp, Giardia spp, Trypanosoma spp, Tritrichomonas sp*p etc causing diarrohea. Cryptosporidium is the intracellular protozoa that causing diarroeiatic disease cryptosporidiosis.

Cryptosporidiosis, caused by *Cryptosporidium sp*, is primarily a disease of calves less than 6 month of age and is usually a milder disease compared to other protozoan diseases like coccidiosis. Infective oocysts are passed in feces and are transmitted by oral ingestion. Oocysts readily infect a variety of animals, including humans. Effective treatments are not available, but because the disease is usually mild and self-limited, supportive care, primarily hydration, is important. Control is strict sanitation and quarantine of sick animals. *Cryptosporidium* has been identified as the cause of numerous outbreaks of diarrhoeal illness in human and animals including cattle. While human infections are thought to be derived from animal sources such as cattle, sheep, goat and other animals, it is not yet established if there is any relationship between human and animal genotypes of *Cryptosporidium.* Cryptosporidiosis may lead to high morbidity and mortality rate (Foreyt, 1990; Robertson, 2009). Symptoms of acute cryptosporidiosis include lack of appetite, and weight loss (Geurden *et al.*, 2008). Clinical signs are yellow diarrhoea with or without blood. Animals will show signs of abdominal pain, anemia, anorexia, dehydration, tenesmus, weakness and loss of weight.

Diagnosis of cryptosporidiosis is an important issue for their effective treatment and control. In field level *Cryptosporidium spp* are identified by modified Ziehl-Neelsen staining techniqe.The Ziehl-Neelsen stain also known as the acid fast stain, was first described by tow German doctors; bacteriologist Franz Ziehl (1859-1926) and the pathologist Friedrich Neelsen (1854-1898).It is a special type of staining that are used to identify the *Cryptosoridium spp* *Mycobacterium spp, Nocardia spp, Isospora spp, Cyclospora spp*, fungal hyphe etc.

Cryptosporidiosis in animals has been reviewed by different authors (Angus, 1983; Tzipori, 1983, 1988; Currant and Garcia, 1991; O`Donoghue, 1995; Olson *et al.,* 2003; Ramirez *et al.,* 2004). Infections of domestic and wild animals provide the biggest source of oocysts which are responsible for environmental contamination. In the USA, *Cryptosporidium* is reported to be present in more than 90% of all US dairy farms and 50% or more of all dairy calves will shed detectable number of oocysts (Sischo *et al.,* 2000). Young animals are more susceptible to infection and disease while in adults it is asymptomatic in most cases. Calves are more susceptible shortly after birth and infection has been reported in both dairy and beef calves (Xiao and Herd*,* 1994; Garber *et al.,* 1994; Atwill *et al.,* 1999). The duration of infection is usually short, lasting about two weeks with peak oocysts shedding during the second week of infection (Ongerth and Stibbs, 1989; Xiao and Herd, 1994; Kemp and Wright, 1995; O’Handley *et al.,* 1999; Uga *et al.,* 2000). Clinical signs are usually manifested in calves early age with mild to moderate, pale or yellowish diarrhoea, which is accompanied by mucus. The condition can last for two weeks and alongside dehydration, calves become lethargic and anorexic, contributing to weight loss. They do not respond to antibiotic therapy and in more severe cases, dehydration and cardiovascular collapse leads to mortality (Olson *et al.,* 2003). However, the healthy calves can be subclinically infected which contributes continuous oocyst excretion in faeces (Tzipori, 1988).

*Cryptosporidium* is also an important cause of enteric infection in young calves and severe outbreaks with high case fatality have been reported by several authors (Tzipori *et al.,* 1981; Angus *et al.,* 1982; Johnson *et al.,* 1999). Previous reports indicate the prevalence of Cryptosporidium from 5% up to 77% worldwide (Bomfim *et al.,* 2005; Ryan *et al,* 2005a; Santin *et al,* 2007). In one report by Geurden *et al.* (2008). Although a number of human cases of cryptosporidiosis have been well documented in childrens and adult diarrheal patients in Bangladesh, there is no report available about the prevalence and speciation of the *Cryptosporidium* sp. responsible for diarrhoea in calves in the country.

Considering the limited data on prevalence of *Cryptosporidium* especially in cattle calves, the present study was undertaken with a number of objectives as below :-

* To identify the oocysts of *cryptosporidium spp.* in cattle calves by Ziehl-Neelsen technique under microscope.
* To investigate the different risk factors of cryptosporidiosis in selected areas of Bangladesh.

**CHAPTER 2**

**REVIEW OF LITERATURE**

**2.1. *Cryptosporidium* and Cryptosporidiosis**

*Cryptosporidium spp.* is member of the phylum Apicomplexa and is found in human and animal populations worldwide. People from both developed and developing countries are vulnerable to these important opportunistic protozoa. The protozoa have a wide host range as it can infect more that 152 species of human and animals. It has a predilection for epithelial cells in the digestive tracts of a wide variety of hosts which includes humans, livestock, companion animals, wildlife, birds, reptiles and fishes (O’ Donoghue, 1995).

**2.1.1. Taxonomy**

Although the first report of *Cryptosporidium* infection in mice was published by Tyzzer in 1907, it was not until 1980s when it was reported as a cause of death in AIDS patients. The earliest cases of human cryptosporidiosis were diagnosed in animal handlers. All species of *Cryptosporidium* are taxonomically classified as below (Levine 1985)-

Phylum: Apicomplexa

Class: Sporozoasida

Subclass: Coccidiasina

Order: Eucoccidiorida

Suborder: Eimeriorina

Family: Cryptosporidiidae

Genus: *Cryptosporidium*

Members of this protozoan genus in the phylum Apicomplexa were long thought to be closely related to the coccidia, important parasites in human and veterinary medicine. Despite strong morphological similarities to the coccidia throughout the life cycle and the presence of mitochondrion-specific genes (Riordan *et al.,* 1999), it has not been shown that *C. parvum* possesses a mitochondria-like organelle (Tetley *et al.,* 1998) as found in classical coccidia. Between 1968 and 1981, different species of *Cryptosporidium* in fish, reptiles, birds, and mammals were named on the assumption that each host species harbored a separate species of *Cryptosporidium* (Fayer *et al.,* 1986; Xiao and Fayer, 2008). While it is not yet confirmed the actual number of species of *Cryptosporidium,* Table 1 shows a list of named species as reported by Xiao and Fayer (2008).

**Table 1. Named Species of *Cryptosporidium* (according to Xiao and Fayer 2008)**

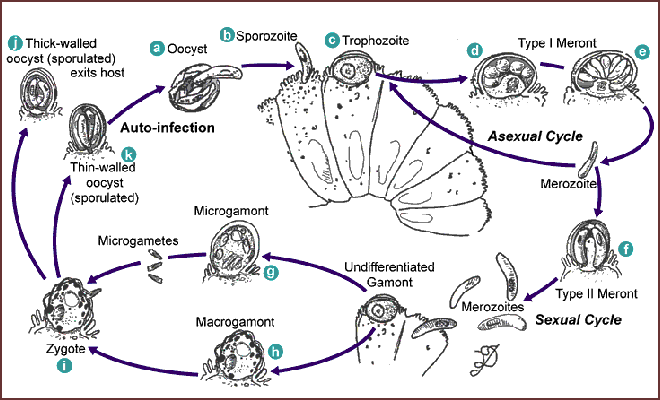
|  |  |  |
| --- | --- | --- |
| **Species** | **Host** | **Author** |
| *C. andersoni* | *Bos Taurus* (domesticated cattle) | Lindsay *et al.,* 2000 |
| *C. bovis* | *Bos Taurus(domesticated cattle)* | Fayer et al,2005 |
| *C. anserinum* | *Anser anser* (domestic goose) | Proctor and Kemp, 1974 |
| *C. baileyi* | *Gallus gallus* (domestic chicken) | Current *et al.,* 1986 |
| *C. agni* | *Ovies aries*(sheep) | Barker and Carbonell, 1974 |
| *C. crotali* | *Crotalus confluens* (snake) | Triffit, 1925 |
| *C. ctenosauris* | *Costa rican* lizard | Duszynski, 1969 |
| *C. cuniculus* | *Oryctolagus cuniculus* (rabbit) | Inman and Takeuchi, 1979 |
| *C. felis* | *Felis catis* (domestic cat) | Iseki, 1979 |
| *C. garnhami* | *Homo sapiens* (man) | Bird, 1981 |
| *C. lampropeltis* | *Lampropeltis calligaster* (lizard) | Anderson *et al.,* 1968 |
| *C. meleagridis* | *Meleagris gallopavo* (turkey) | Slavin, 1955 |
| *C. muris* | *Mus musculus* (domestic mouse) | Tyzzer, 1907 |
| *C. nasorum* | *Naso literatus* (fish) | Hoover *et al.,* 1981 |
| *C. parvum* | *Mus musculus* (domestic mouse) | Tyzzer, 1912 |
| *C. rhesi* | *Macaca mulatta* (rhesus monkey) | Levine, 1981 |
| *C. serpentis* | Colubrid, crotalid, and boid snakes | Levine, 1981 |
| *C. tyzzeri* | *Gallus gallus* (domestic chicken) | Levine, 1961 |
| *C. vulpis* | *Vulpes vulpes* (European fox) | Wetzel, 1938 |
| *C. wrairi* | *Cavia porcellus* (guinea pig) | Vetterling *et al.,* 1971 |
| *C. andersoni* | *Bos Taurus* (domestic cattle) | Lindsay *et al.,* 2000 |
| *C. bovis* | *Bos Taurus* (domestic cattle) | Fayer *et al.,* 2005 |
| *C. canis* | *Canis familiaris*(domestic dog) | Fayer *et al.,* 2001 |
| *C. fayeri* | *Macropus rufus* (red kangaroo) | Ryan *et al.,* 2008 |
| *C. galli* | *Gallus gallus* (chicken) | Pavlasek 1999 |
| *C. hominis* | *Homo sapiens* (human) | Morgan-Ryan *et al.,* 2002 |
| *C. suis* | *Sus scrofa* (domestic pig) | Ryan *et al.,* 2004 |
| *C. xiaoi* | *Ovis aries* (domestic sheep) | Fayer and Santin, 2009 |

**2.1.2. Morphology**

Among the coccidia, the genus *Cryptosporidium* has the smallest oocysts. They are spherical to ovoid and 50 fully sporulated oocysts averaged 7.4 by 5.6 µm for *C. muris* and 5.0 by 4.5 µm for *C. parvum*, the species infectious for most mammals (Upton *et al.,* 1985). Sporulated oocysts each contain four sporozoites and a residuum composed of numerous small granules and a spherical or ovoid membrane-bound globule. Most, but not all, authors report no sporocyst wall within the oocyst (Levine, 1984). Other morphological features often observed in coccidian oocysts, such as a micropyle and polar granules, have not been found in oocysts of *Cryptosporidium spp*. The oocyst wall is smooth and colorless and averages about 50 µm in thickness. It is composed of two electron-dense layers separated by a thin electron-lucent space (Reese *et al.,* 1982). The small size of the *Cryptosporidium* oocysts makes them indistinguishable at the species level based on morphology by light microscope (Fall *et al.,* 2003). The oocysts are spherical or ovoid in appearance and contain 4 naked parallel sporozoites surrounded by a smooth oocyst wall. At the wall, a faint suture can be seen through which the sporozoites exit during excystation (Morgan-Ryan *et al.,* 2002). There is some variation from species to species. The length of the oocyst ranges from 4.5 to 7.5 μm and the width from 4.2 to 5.7 μm.

**2.1.3. Life cycle**

The life cycle of *C. parvum* has been outlined in a number of reviews (Fayer and Ungar, 1986; Current and Garcia, 1991; O’Donoghue, 1995). *Cryptosporidium* is monoxenous, that is, its life cycle is completed within one host. The parasite moves from host to host via the faecal-oral route (Fayer and Ungar, 1986). The lifecycle (Fig. 1) begins with the ingestion of oocysts which excysts in the intestine releasing sporozoites. Thereafter, two cycle of schizogony is followed by gametogony with the production of male and female gametocytes (Tzipori and Griffiths, 1998). The trophozoites within parasitophorous vacuole undergo asexual multiplication by schizogony with production of merozoites. Several investigators reported endopolygeny for multiple division and sequential development involving two types of meronts (Vetterling *et al.,* 1971a; Iseki, 1979; Current & Reese, 1986). Type I meronts form 8 merozoites, which are released when mature and are termed as type I merozoites. The type- I merozoites then invade other noninfected epithelial cells where they undergo another cycle of merogony and develop into type II meronts. The type II meronts form 4 type II merozoites, which do not undergo further merogony but produce macro and microgamonts.



**Fig-1. Different life cycle stages of *Cryptosporidium spp (Reproduced from*** [***www.*sciencedirect.com**](http://www.sciencedirect.com/)**).**

Sexual reproduction occurs by gametogony and both microgamonts and macrogamonts are formed from type II merozoites (Gobel & Brandler, 1982). Microgamonts develop into microgametocytes, which produce up to 16 non-flagellated microgametes. Macrogamonts develop into macrogametocytes and are fertilized by mature microgametes. The product of fertilization is zygote, around which a resistant wall is formed and subsequently termed as “oocyst”. Most oocysts are thick-walled and are excreted from the host in faecal material.Approximately 20% of the oocysts produced in the gut fail to form an oocyst wall and the developing sporozoites are only surrounded by a series of membranes. These "oocysts," devoid of a wall, are called "thin-walled oocysts."

The resultant zygotes undergo further asexual development (sporogony) and through meiosis lead to the production of sporulated oocyst containing 4 sporozoites. The thin walled oocyst is responsible for autoinfection within the host, which have been reported to excyst within the same host animal leading to a new cycle of development (Current, 1985; Current & Reese, 1986). Thus the thin walled oocysts and recycling type-I meronts are responsible for persistent chronic infection in the same host. The thick walled oocyst containing four sporozoites is ingested by the host from the environment, which are released into the epithelial cells of intestine where asexual development starts (Tzipori and Griffiths, 1998).

**2.1.4. Diagnosis, treatment and control of cryptosporidiosis**

**2.1.4.1 Diagnosis**

A number of tests have been developed for the diagnosis of *Cryptosporidium*. These involve direct detection by microscopy of faecal materials after using specialized staining techniques (Garcia *et al.,* 1983). The modified acid-fast stain (Ziehl-Neelsen stain) and auramine stain is widely used but has the limitation of relatively low sensitivity (Weber *et al.,* 1991). However, examining under UV light with a rhodamine filter can increase the sensitivity up to 100 times (Nielsen and Ward, 1999). Use of polyclonal or monoclonal antibodies for detection by immunolabelling has also been developed, but proved less sensitive than conventional staining (Garcia and Shimizu, 1997). The recently developed PCR amplification technique has been found to be very specific and highly sensitive, targeting different genes including the oocyst wall protein (COWP), the small subunit of rRNA, -tubulin, TRAP-C1, TRAP-C2, ITS1, polythreonine repeat (poly-T), dihydrofolate reductase (DHFR), and sequences and mRNA of heat shock proteins (Sulaiman *et al.,* 1999). The efficacy of this technique for detection of *Cryptosporidium* in environmental and clinical samples has also been reported in several studies (da Silva *et al.,* 1999). Certainly, the use of the PCR technique with sequence analysis provides help for genetic characterization of *Cryptosporidium* at the species level which can contribute significantly to epidemiological investigations.

**2.1.4.2. Treatment**

Despite intensive efforts, treatment of cryptosporidiosis with available anticoccidials is still not satisfactory (Haberkorn, 1996; Coombs and Muller, 2002). The macrolides, spiramycin and azithromycin have been found ineffective and less tolerable in both immunocompetent and immunodeficient individuals (Saez-Llorens *et al.,* 1989; Galvagno *et al.,* 1993; Vargas *et al.,* 1993; O’Donoghue 1995). Diclazuril was tested in humans with unsatisfactory results (Connolly *et al.,* 1990; Soave, 1990). In AIDS patients, letrazuril induces clinical improvements in up to 50% of patients and some inhibition of oocyst excretion (Guillem *et al.,* 1992; Harris *et al.,* 1994). Unfortunately, none of the widely used anticoccidials are suitable to treat clinically infected patients. Thus the search for new anticoccidials to treat cryptosporidiosis continues.

**2.1.4.3. Control**

Prevention is the most effective approach to control cryptosporidiosis. Contamination of water sources is the major source of human infection and thus prevention of environmental spread of oocysts is crucial. Cattle farms should be constructed away from streams and rivers to avoid possible water contamination. Prophylactic measures should be taken to reduce the transmission between animals, as they are the main source of zoonotic infection. This involves effective herd management without overcrowding or reducing stocking density, treatment of infected cattle separately, keeping young animals from the adults and minimizing human contact with calves (Ramirez *et al.,* 2004). The destruction of oocysts with 5% ammonia solutions with heat is recommended for cleaning houses (Campbell *et al.,* 1982).

**2.1.5. Cryptosporidiosis in large ruminants**

The parasite is common in ruminants throughout the world but reported prevalence rates vary widely. Among calves up to 100% of a herd may be affected, especially if the animals are housed communally (Casemore *et al.,* 1997; de Graaf *et al.,* 1999; Joachim *et al.,* 2003; Sturdee *et al.,* 2003).In India 461 calves fecal and 264 buffalo calves are taken and found the prevalence is 16.3% in Cattle calves and 24.5% in buffalo calves.In middle Egypt 458 fecal samples are collected and 14.9% positive case are found in Cryptosporidiosis positive (Anderson 1982; Angus *et al.,* 1982; Barker 1974; Berg *et al.,* 1978; Ducatelle *et al.,* 1983; Hiepe *et al* 1985; Tzipori *et al.,* 1981) Calves at 1-15 days were at the highest risk (P < 0.001), and a significant relationship between season and infection (P < 0.05) was recorded. A significant association between infection and hygiene (P < 0.001), type of floor (P < 0.01) and source of water (P < 0.01) was also recorded. Statistical analysis concerning the clinical signs and fecal characteristics revealed a significant association with fecal consistency (P < 0.001), presence of blood (P < 0.01) and mucous (P < 0.01). Moreover, a significant association was found between infection and the desire for suckling (P < 0.05) and tenesmus (P< 0.05). The results of the present study demonstrated the strong relation between infections by *Cryptosporidium spp.* and diarrhea in buffalo calves(Osama et al.,2008; Neonates are most susceptible to natural infections (Anderson 1982; Angus *et al.,* 1982; Barker 1974; Berg *et al.,* 1978; Ducatelle *et al.,* 1983; Hiepe et al 1985). Experimental studies have shown that older calves also were susceptible to infection, 30-dayold calves were infected but had only a mild clinical response, and calves as old as 7 months were infected but no clinical information was provided (Tzipori *et al.,* 1981). Diarrhoea, the most prominent clinical sign of bovine cryptosporidiosis, lasted 2 to 12 days and was sometimes accompanied by anorexia, poor growth, stiffness, hyperpnea, slow gait, limb muscle fasciculations, and depression. Most cases were diagnosed by identification of oocysts in feces. At necropsy, blood or mucoid fluid and bright yellow watery feces have been found in the colon, and both the small and large intestine have appeared mildly hyperemic (Berg *et al.,* 1978, Tzipori *et al.,* 1981).

**2.1.6. Cryptosporidiosis in Bangladesh**

Cryptosporidiosis has long been considered as an important pathogen causing diarrhoea in Bangladesh (Shahid *et al*., 1987). The very first report of cryptosporidiosis in Bangladesh indicates possible zoonotic transmission as reported from calves, animal handlers and associated family members at a dairy farm in Savar (near the capital city, Dhaka) (Rahman *et al.,* 1990).

A prospective study on the urban slum in capital city, Dhaka reported that malnutrition significantly increases the risk of cryptosporidiosis along with some enteropathogen (Mondal *et al.*, 2006). In another study, association of enteric protozoan-associated diarrheal illness with that of the nutritional status and growth of preschool children in Bangladesh was investigated. While comparatively more childrens were suffering from infection with *Giardia* and *Cryptosporidium spp*, no relationship was found between malnutrition and stunting growth of calves with cryptosporidiosis (Mondal *et al.,* 2006).

Since long time, diarrheal diseases were considered as a leading public health problem, particularly in children in Bangladesh. Early studies in rural Bangladesh also indicated persistent diarrhoea in childrens as a concern for public health (Huttly *et al.*, 1990). A separate recent study also indicated that *E. histolytica, C. hominis, C. parvum, and G. lamblia* assemblage A infections are important causes of diarrhoeal illness in Bangladesh population. The prospective case-control study was performed which involved a total of 3,646 case patients and 2,575 control subjects with asymptomatic infection (Haque *et al.*, 2009).

Recently modern molecular biological approaches like multiplex real-time PCR assay has been used experimentally to identify different etiologic agents of diarrhoea including *Cryptosporidium* spp. in Bangladesh. However it is expensive to be used as routine diagnostic tool throughout the country (Haque *et a.*, 2007). Using scorpion probes and real-time qPCR based on 18s rRNA gene, several *Cryptosporidium* species were reported from stool samples originated from Bangladesh. These include *C. parvum, C. hominis, C. felis and C. meleagridis* (Stroup *et al.,* 2006).

Few studies have been directed to investigate the epidemiology, clinical features, and systemic antibody responses of cryptosporidiosis in Bangladeshi children. In one such study by Khan *et al.* (2004), *Cryptosporidium* spp. infection was found to occur most commonly in those childrens who are less than two years of age and was accompanied by watery diarrhoea and vomiting. In addition, *Cryptosporidium*-specific serum IgM levels were reported as significantly higher in cases compared with controls in the study groups.

The epidemiology, clinical features, nutritional status, and causative agents of diarrhea in Bangladeshi children were studied by Haque *et al*., (2003). Among other bacterial and viral pathogens *Cryptosporidium* was reported from 8.4% (n=893) samples as was identified as important cause of diarrhoea among childrens. A year long surveillance in a hospital at Dhaka, Bangladesh identified 1.4% (n=814) cases of diarrhoea caused by *Cryptosporidium spp*. in children under 5 yrs of age (Albert *et al.*, 1999). Another year-long study from the same hospital identified 3% (n=1382) incidence of *Cryptosporidium* oocysts in the diarrhoeal stool samples (Rahman *et al.*, 1990).Also a study had occurred in CVASU by Maqsudul Alam reported that 15 positive cases are found out 100 no. of sample in goat kids sample.(Maqsud et al.,2012)

**CHAPTER 3**

**MATERIALS AND METHOD**

**3.1. Source of samples**

During the study, a total of 50 fresh fecal samples were collected from dirrhoeic cattle calves of different region of pabna, sirajgong and Chittagong district and calves arriving at SA Quadery Teaching Veterinary Hospital (SAQTVH) of the Chittagong Veterinary and Animal Sciences University (CVASU). The ages of the animals were between 1 to 6 months and samples were collected by visiting different type of farm. All relevant data such as breed, sex, age, housing, feeding, treatment history were recorded for further analysis.

**3.2. Study area and period**

All the animals were located in different area of Bangladesh like Sirajgong, Pabna and around Chittagong Metropolitan area (CMA). The experiment was carried out at the Animal Disease Diagnostic Lab (ADDL) of Poultry Research & Training Center (PRTC) and the Parasitology Laboratory of the Department of Pathology & Parasitology, Chittagong Veterinary & Animal Sciences University (CVASU). The study period was during September, 2013 until March, 2014.

**3.3. Collection of fecal samples**

Feces were collected directly from the rectum of each cattle calf into a clean plastic specimen container that was immediately come to preservation in freezer, then I labbled the container and transport to Chittagong through icebox. The fecal samples were immediately used for staining or preserved in – 20°C freezer.

**3.4. Ziehl-Neelsen staining technique**

This is technique is used for the detection of oocysts of *Cryptosporidium* species in faeces. (Henricksen and Pohlenz,1981).

**3.4.1. Materials and reagents**

**3.4.1.1. Materials required**

* Fecal samples
* Slides
* Coplin jar
* Electric drier
* Compound microscope
* Immersion oil
* Tooth pick
* Cover slip

**3.4.1.2. Reagents**

* Methanol
* 3% carbol fuchsin
* 1% acid methanol
* 0.4% brilliant green

**3.4.1.3. Reagents composition**

* Recipe for 3% carbon fuchsin:

3 gm basic fuchsin

30 ml ethanol

15 ml carbolic acid

Up to 100 ml distill water

* Recipe for 1% acid alchol

70% Methanol 99 ml

Concentrated HCL 1 ml

* Recipe for Brilliant green

Brilliant green powder 0.4 gm

Up to 100 ml distill water

**3.4.2. Procedure**

* Using the tooth pick, a medium to thick smear of fecal materials were prepared and air- dried.
* The dried smear was then flooded with methanol for 3 min for fixation and then air-dried
* Later 3% carbol fuchsin was poured over the smear and left for approximately 15 min
* The slide was rinsed with running tap water
* Now 1% acid methanol was added in the smear to decolorise and left for 15 to 20 seconds
* The slide was again rinsed with running tap water
* Now 0.4% brilliant green was poured to counterstain and left for 30 seconds
* The slide was again rinsed with running tap water and then air-dried
* The stained slide was then examined using a compound microscope

**3.4.3. Microscopic examination**

Air dried slides were examined under light microscopy at X10, X40 and X100 magnifications. Ocyst 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 brilliant green (Fig.7 and 17).

Fig2. Feces samples of Calves Fig 3. Reagents used in Z-N technique

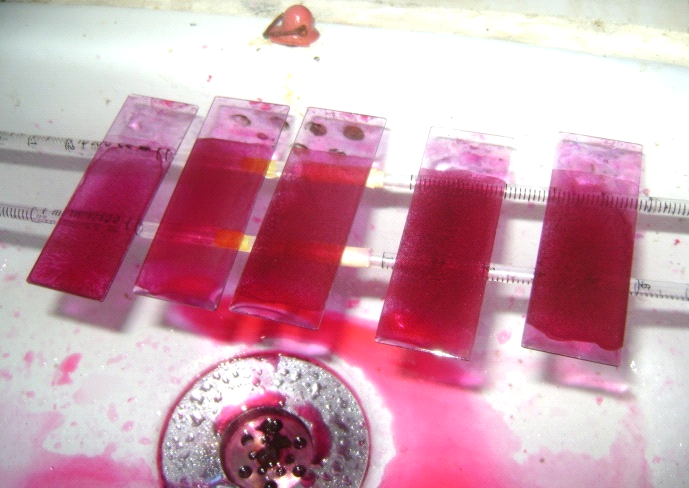
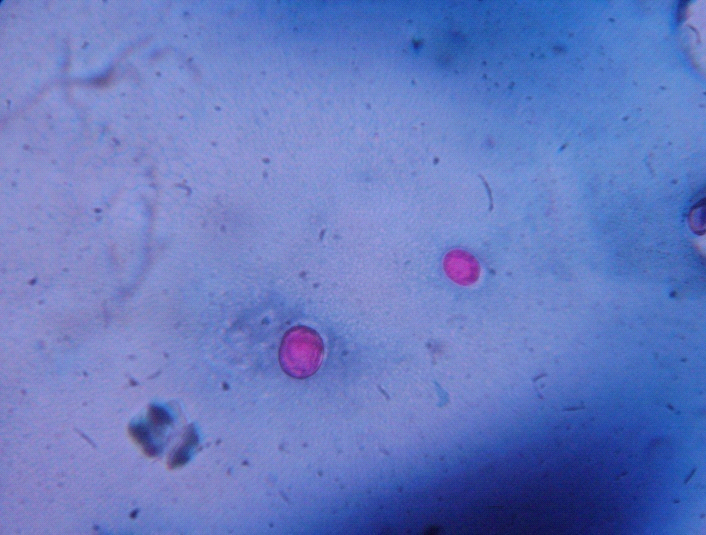
 

Fig 4. Fecal smear dried in drier. Fig 5. Smear staining with carbol

fuchsin.

 Fig 6. Smear staining with brilliant Fig 7. Oocysts under microscope

green

**CHAPTER 4**

**RESULTS**

**4.1. Microscopic identification**

Initially after sampling the feces from diarrhoeic cattle calves, classical tools like Ziehl-Neelsen stain was used to identify *Cryptosporidium* positive cases. Through microscopy, only 5 sampleswere found as positive and oocyst was found (fig:8a,8b) out of 50 samples tested.

During this study four different types of breeds of cattle are taken namely Jersy cross, Holstein-Friesian cross,RCC and undescribed breed were sampled. While all of the calves were having diarrhoea, it was notable that Cattle calves out of the 5 cases were those of Holstein-Friesian and undescribed breed was found positive based on Ziehl-Neelsen staining and microscopy.

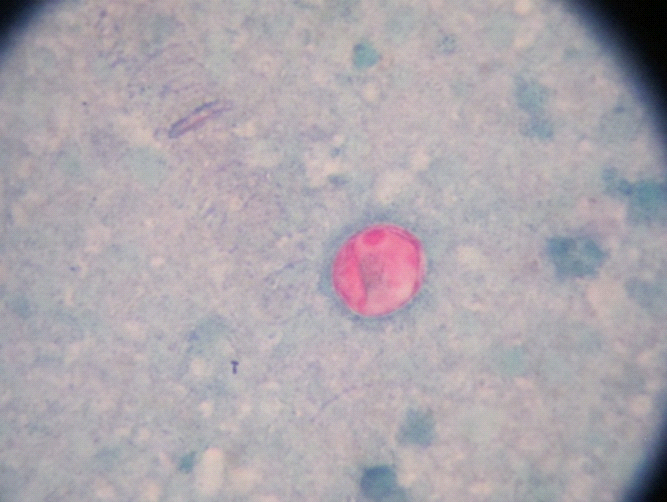
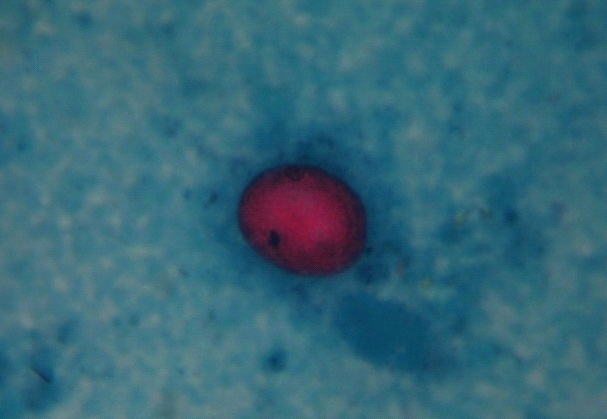
 

Fig 8 (a) and (b). Oocysts of *Cryptosporidium sp*. in modified Ziehl-Neelsen stain under microscope in **×**100 objectives.

Fig 9. Breed specific prevalence of cryptosporidiosis

Fig 10. Sex specified prevalence of Cryptosporidiosis

**CHAPTER 5**

**DISCUSSION**

Cryptosporidiosis in calf have been reported in several countries by a number of investigators. The incidence and prevalence rates have been reported that varies widely according to the sample size and geographic distribution. The prevalence recorded during this study shows that 10% in Cattle calves (5 out of 50) was positive for *Cryptosporidium***.** This finding was based on microscopic examination by Ziehl-Neelsen stain which is not 100% confirmatory. This is because not only cysts of *Cryptosporidium* but other organisms might interfere with the the test results. These includes several acid fast bacteria including *Mycobacterium sp.*  This rate of prevalence was somewhat comparatively low with other investigators such as 23% in UK (Sturdee et al., 2003), 24% in Romania, 40-70% in Spain (Munoz *et* *al.*, 1996; Casemore *et al.*, 1997; Castro-Hermida *et al.*, 2002; Causapé *et al*., 2002). Again this was comparatively higher as in Belgium where cryptosporidiosis was reported only in 9.5%. However, the rate was 20% in Trinidad and Tobago and 17.6% in Iran which indicates that further study can ensure actual prevalence of cryptosporidiosis in calves in Bangladesh. Considering this study as the first of its type, further sampling is warranted from different divisions, districts, farms and household level. It can be postulated that the comparative high rate of infection in these countries is due to high level of environmental contamination in Belgium and Spain which is usually very different in Bangladesh. Most large ruminants like Cattle and Buffalo in Bangladesh have little access to pasture and usually they are reared in semi-intensive method where there is little opportunity for the young animals to be infected by the contaminated oocysts. Due to lack of information in the subcontinent, we were unable to compare the occurrence of cryptosporidiosis in other large ruminants in the Indian subcontinent or Southeast Asia.

Considering the relative susceptibility, our data indicated that undescribed breed of cattle calves are more vulnerable to cryptosporidiosis compared to the exotic breed. This can highlight new thoughts on breed-associated immunologic factors and further research can answer if this can increase our understanding in developing new methods of immunoprophylaxis. A large number of articles have been published on the development of DNA vaccine but still now none was completely successful. Future research should be directed to elucidate breed-specific factors that may lead to consideration of a vaccine against cryptosporidiosis.

Concerning the sex-specific vulnerability, our data showed that female calves were more vulnerable compared to their male counterpart. This could be associated with different hormones that may contribute to variable susceptibility of different calves to this infection. This observation is also important as most of the marginal farmers in Bangladesh prefer rearing female goats and reduction of morbidity and mortality due to cryptosporidiosis would be crucal to help them reducing poverty. Further research can identify the biological factor that is responsible for this sex-specific susceptibility.

**CHAPTER 6**

**CONCLUSION**

Cryptosporidiosis is a moderate type of protozoal disease of animal. Prevalence of Cryptosporidiosis in Bangladesh is vary with different area. In this study we found that 10% fecal samples were positive for *cryptosporidium* *spp* which might be the cause of diarrhea on that claves. In field level diagnostic procedure and its treatment is not satisfactory .But *Cryptosporidium* *spp* have zoonotic significance. For that reason we have to concern about this disease and need more research. Although it is not sufficient for further research from but It is true that this report will help to know primary knowledge about the percentage of cryptosporidiosis in diarrhoiec calves, becuse to our best knowledge this is the first work about cryptosporidiosis of diarrhoeic cattle calves in Bangladesh.

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