Occurrence of *Eimeria* spp. in neonatal calves in Chittagong metropolitan area

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Registration No. 217

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Pathology

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Dedication

To my beloved parents

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This is to certify that we have examined this thesis and have found that it is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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The Author
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List of abbreviations used

Abbreviation and symbol	Elaboration
CVASU	Chittagong Veterinary and Animal Sciences University
DLS	Department of Livestock Services
GDP	Gross Domestic Product
OD	Odd Ratio
OPG	Oocysts per gram of Feces

Summary

Calf diarrhea is a major problem in cattle farming in Bangladesh where coccidiosis is common phenomenon. The specific objective of the present study was to determine the prevalence of coccidia infection in neonatal calves as well as to assess the risk factors associated with the infection. During the study, fecal samples were collected from 130 calves (less than 45 days of age) from a total of 39 dairy and beef farms located in Chittagong Metropolitan Area and Patiya Upazila of Chittagong. The study was conducted during June, 2015 to December, 2015. The prevalence of *Eimeria* spp. was found 20.77% in diarrheaic calves in the study area. 26% and 27% prevalence of *Eimeria* spp. was found considering age in calves between 2-4 weeks and > 4 weeks, respectively. Univariable and multivariable logistic regression models were used to evaluate the risk factors associated with *Eimeria* spp. infection. In univariable logistic regression analysis, 5 variables (age, flooring of calving pen, source of drinking water, fecal consistency and dehydration test) showed significant (P-value <0.2) association with the prevalence of *Eimeria* spp. In multivariable analysis, age and fecal consistency were found significantly (P-value <0.05) associated with presence of *Eimeria* spp. Calves with more than 4 weeks of age and calves with 2 to 4 weeks of age had odds ratio of 9.03 and 6.58, respectively compared to calves with less than 2 weeks of age. Calves with liquid (OR=4.5) and semi-solid (OR=1.4) fecal consistency had higher chance to get the infection compared to calves with solid feces. The results of the present study identified the risk factors associated with occurrence of *Eimeria* spp. causing diarrhea in neonatal calves will be helpful for developing suitable targeted control program in the study area.

Keywords: Calf diarrhea, *Eimeria* spp, Prevalence, Risk factors.

Chapter-1: Introduction

Livestock is a key component of the agricultural economy of Bangladesh. The contribution of the livestock sector to overall GDP has been provisionally estimated at 1.66% for 2015-16 (DLS, 2016). Despite its modest share of overall GDP, livestock serves an essential role as a source of protein, employment generation, export earning, and provision of food security. Current cattle population in Bangladesh is 237.85 lakh while it is not sufficient compared to annual demand for milk, meat and other by-products for the extensively large population. Shortage of livestock products like milk, meat demands more extensive cattle farming. However, several factors hinder the animal rearing substantially among which outbreak of disease is considered as the major cause.

Rearing young stock is an important part of farm management. During this early phase of an animal's life, it is very susceptible for all kinds of infections causing disease or even death. Intestinal and respiratory disorders are common problems which cause reduction of the health status of calves. (Svensson *et al*., 2006; Gulliksen *et al*., 2009). Intestinal problems are often associated with diarrhea, which leads to weight loss, reduced growth and a higher first calving age, all resulting in economic losses. (Lorenz *et al*., 2011). Diarrhea is a very common symptom of digestive disorder that occurs due to a lot of causes. Diarrhea can be caused by viruses, bacteria, protozoa and is favored by factors associated with housing and hygienic conditions (De Graaf *et al*., 1999; Lorenz *et al*., 2011).

Important infectious agents causing diarrhea are Rotavirus, Coronavirus, enterotoxigenic *E. coli, Salmonella* spp., *Cryptosporidia* spp. and *Eimeria* spp., either singly or in combination (Steiner *et al*., 1997; De la Fuente *et al.*, 1998).

Coccidiosis is a protozoal disease in calves caused by *Eimeria spp.* that cause severe diarrhea in young animals (Bangoura *et al*., 2012). Clinical disease is characterized by bloody scours or red diarrhea, dysentery, weight loss and death. The disease only occurs if an animal is subjected to heavy infection or its resistance is lowered. The presence of infection does not invariably lead to the development of clinical signs of disease. Low level of challenge can actually be beneficial by stimulating protective immune responses in the host (Catchpole *et al.*, 1993).

Coccidiosis is a common problem in calves in general. Studies have shown high prevalence of *Eimeria* oocyst excretion of 8% up till 100% at cattle farms in general (Bangoura *et al*., 2012). For example, prevalence of *Eimeria* spp. oocysts in Pakistan was estimated as 47.09% (Rehman *et al*., 2011). To identify risk factors which contribute to the *Eimeria* infection, a lot of factors have been mentioned in literature. Risk factors like larger herd size, having a non-slatted floor, poor hygienic conditions, poor climatic conditions in the stable, high stress levels of the calves and high animal density are mentioned to contribute to a higher oocyst excretion of *Eimeria* (Rehman *et al*., 2011; Koutny *et al*., 2012; Bangoura *et al*., 2012). Few studies carried out previously to determine the gastrointestinal parasite prevalence in Chittagong district. However, to our knowledge, there is no prior studies about the prevalence and risk factors associated with bovine coccidiosis in Chittagong district.

By knowing the burden of *Eimeria* spp. infection in diarrheaic calves and corresponding risk factors in cattle farms, efficient and targeted control program can be executed in prevalent areas. The information will also update the knowledge of attribution of diarrhea to different microorganism. Overall, the study result will lead to improvement of young stock and reduce economic losses in cattle farms due to coccidiosis.

The aim of this study was therefore

1. To determine the prevalence of calf diarrhoea attributed to *Eimeria* spp. in Chittagong Metropolitan Area.
2. Determination of risk factors associated with *Eimeria* infection in calves.

Chapter-2: Review of Literature

2.1. Calf Diarrhea

Diarrhea in young pre-weaned calves is one of the most common phenomena in cattle worldwide. Calf diarrhea is a general term that refers to a disease complex characterized by acute, undifferentiated diarrhea and caused by a number of infectious (bacteria, viruses, parasites) pathogens (Izzo *et al.*, 2011). It attributes moderate to severe adverse effect on the calf's health status, longevity in the herd, productivity performance and even death and thus causes great economic losses. Despite infectious agent's other factors including age, farm management, herd size and environmental factors, may also influence the clinical outcome (Rehman *et al*., 2011). Bovine coccidiosis caused by *Eimeria* spp. primarily causing varying degree of bloody diarrhea, dehydration, severe intestinal lesions, and sometimes even death (Cho and Yoon, 2014).

2.2. Infectious etiology of calf diarrhea

Numerous infectious agents (bacteria, viruses, parasites) are involved in calf diarrhea. According to Cho and Yoon, (2014) major enteric pathogens causing diarrhea are bovine rotavirus, bovine coronavirus, bovine viral diarrhea virus, *Salmonella* spp., *E. coli*, *Clostridium perfringens* and *Cryptosporidium parvum* while calf diarrhea caused by *Eimeria* spp. is considered as a ubiquitous agent in cattle rearing (Daugschies and Najdrowski, 2005).

Escherichia coli is a primary cause of calf diarrhea. It is a gram-negative, facultative anaerobe and nonspore forming member of the Enterobacteriaceae family. Based on virulence scheme *E. coli* can be classified into six pathogroups (enteropathogenic (EPEC), enterotoxigenic (ETEC), attaching and effacing (AEEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC) or shiga toxin producing *E. coli* (STEC). Neonatal calves are most susceptible to Enterotoxigenic *E. coli* (ETEC) and shiga toxin producing *E. coli* (STEC) causing profuse watery diarrhoea (Jay *et al.*, 2004).

Salmonella sp. is gram-negative, non-spore-forming facultative anaerobe and motile bacteria causing diarrhea. They have several serotypes including *S. enterica serovar Typhimurium* (*S. typhimurium*) and serovar *Dublin* (*S. dublin*), *S. muenchen* and *S. Copenhagen* causing salmonellosis in cattle. *S. typhimurium* is the most common serotype that affects calves (Tsolis *et al.*, 1999).

Clostridium perfringens is a Gram-positive, spore forming anaerobic bacterium that causes diseases by producing five major toxins (alpha, beta, epsilon, iota and enterotoxin). Based on this virulent toxin production they can be divided into five types (A, B, C, D and E). Calves are easily infected by *C. perfringens* type C due to low level of proteolytic enzyme like trypsin in their digestive tract. Diffuse or multifocal hemorrhagic necrotizing enteritis and bloody fluid distension are characteristic lesions found in *C. perfringens* infection (Barker *et al.*, 1993).

Bovine rotavirus is considered as a common cause of enteric illness in the newborn calves. Rotavirus belongs to the Reoviridae family possesses 11 double-stranded RNA segments (16∼21 kb) enclosed in a triple-layered capsid. Although they usually cause high morbidity, most rotavirus infections are mild and self-limiting (Maclachlan and Dubovi, 2011).

Bovine Coronavirus (BCV) causes neonatal diarrhoea in calves aged between 3-21 days. Viral replication occurred in the epithelium of the villi of the small intestine and cryptal colonic epithelium that causes severe enterocolitis (Decaro *et al.*, 2008).

Bovine viral diarrhea virus (BVDV) belongs to the genus Pestivirus under the family Flaviviridae (ICTV, 2011). The virus is classified into two types non-cytopathogenic (ncp) and cytopathogenic (cp) based on the what effects they produced on the bovine cell culture (Mockeliuniene *et al.,* 2004). BVD virus occasionally cause severe diarrhoea in neonatal calves (Campbell *et al*., 2004).

Cryptosporidium spp. are protozoan parasite that causes infectious diarrhoea among young farm animals. *C. parvum, C. bovis, C. ryanae, and C. andersoni* are the common infectious agent among approximately 24 species of *Cryptosporidium* that infect cattle. *C. parvum* usually infect calves between 1 and 4 weeks of age as well as humans (Kvac *et al*., 2006).

2.3. Calf diarrhea caused by *Eimeria* spp.

Bovine coccidiosis (known as red diarrhea) is produced by various species of *Eimeria*. The most pathogenic are. *E.bovis* and *E.zuernii*. and infection with *Eimeria* sp. is a common cause of diarrhea in calves usually known as bovine coccidiosis has been reported worldwide. More than 20 different species of *Eimeria* have been documented

until now that infect cattle and buffalo (Enemark *et al*., 2013). Prevalent species of *Eimeria* are *Eimeria bovis, Eimeria zuernii, Eimeria auburnensis, Eimeria anadensis, Eimeria ellipsoidalis, Eimeria subspherica, Eimeria cylindrica, Eimeria alabamensis, Eimeria wyomingensis, Eimeria bukidnonensis, Eimeria illinoisensis, Eimeria pellita, and Eimeria rasilensis* (Sanchez *et al*., 2008; Abebe *et al*., 2008). *E. bovis* and *E. zuernii* are the most prevalent and highly pathogenic agents that cause clinical disease in calves. In contrast, rests of the *Eimeria* spp. are considered as low pathogenic to animals (Daugschies and Najdrowski, 2005).

2.4. Parasite biology

The life cycle of *Eimeria* spp. is monoxenous with an endogenous (schizogony and gametogony) and exogenous (sporogony) phase (Figure:1) (Daugschies and Najdrowski, 2005). The transmission is fecal-oral route from contaminated water and feed.

Upon ingestion of the infective oocyst by healthy animal, the sporozoites are released into the intestinal lumen due to stimulation by carbon dioxide, trypsin and bile. This process is called 'excystation'. Individual sporozoites then invade the intestinal mucosal cells by the release of antigens from organelles (micronemes and rhoptries) that are important for host cell recognition, penetration through host cell membrane and parasitophorous vacuole formation. Within parasitophorous vacuole sporozoites transform into a round structure called 'trophozoite' and become larger to develop into schizont. Mature schizont releases the slender shaped active merozoites following rupture that further penetrate new cells and repeat the process to produce second generation merozoites (Daugschies and Najdrowski, 2005).

After the second generation of schizogony the merozoites entering new cells differentiate into microgamont (male) and macrogamonts (female). Then fertilization of the macrogamete occurs by one of the microgametes resulting formation of oocyst which are then shed with the feces to the environment (Coetzer and Justin, 2004).

Oocyst sporulation takes place depending on proper moisture, temperature and other environmental factors. During this sporulation, oocyst cytoplasm divides to form four sporocysts, each harboring two sporozoites (Svensson *et al.*, 1994).

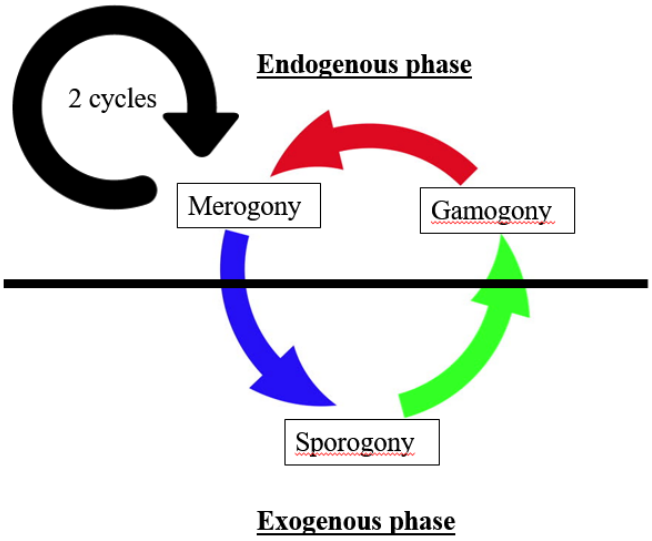

Figure 1: Schematic presentation of the life cycle of *Eimeria* spp.

2.5. Pathophysiology

Eimeria spp. are obligate, intracellular parasites develop within the cytoplasm of intestinal epithelial cells. The effect on the host depends on the magnitude of the initial infective dose and the number of cells invaded by the sporozoites. The effect also depends on the spread of infection during schizogony. As increasing numbers of organisms enter sexual phase (gametogenesis) infection of new cells by merozoites declines and the disease gradually subsides.

Disease severity depends on several factors including the number of oocysts ingested. Oral administration of 50,000 oocysts of *E. bovis* to naive calf causes diarrhoea, while 100,000 results severe hemorrhagic scours (Daugschies *et al*., 1986). When large number of the intestinal epithelium are parasitized intense inflammation is observed in lamina propria together with submucosa. In response to this pathologic condition, hyperplasia of the intestinal epithelium is eventually happened as body homeostasis. *Eimeria* gametogenesis is most numerous in that the lesion exhibiting this hyperplasia (Daugschies and Najdrowski, 2005).

Detail histopathological sequels of *E. bovis* infection have been described by Friend and Stockdale (1980). They stated that sexual stages produce huge destruction of intestinal mucosa as enormous reproduction of the parasites leads to increasing number of destructed intestinal cells. Consequently, scattered fibrin strands with edematous caecal submucosa followed by diptheric enteritis were found in the caecum, colon, and

the distal part of large intestine. Catarrrhalic to haemorrhagic diarrhoea develop depending on the severity of the lesions.

Different species of bovine coccidia localize in different parts of intestine. *E. zurnii* and *E. bovis* occur mainly in the caecum, colon and last part of the ileum, whereas, *E. alabamensis* is occurring within the nuclei of the epithelial cells at the tips of villi (Svensson *et al.*, 1994).

In *E. bovis* infection, the later stages of the first generation schizont cause distortion of villi and disruption in the case with the second generation schizont which cause the greatest pathogenic effect. In severe infections majority of the crypts of the large intestine and sometimes the terminal part of the small intestine become destroyed. Denuded epithelial layer and blood filled intestinal lumen is commonly observed. The mucosa is necrotic and sloughed off and this damage may extend to the sub mucosa, the wall of the intestine is congested and edematous, thickened with petechial or diffuse hemorrhages. Large number of gametocytes and oocysts are visible microscopically (Daugschies and Najdrowski, 2005).

Considering pathogenesis on individual basis *E. zurnii* is the most pathogenic, while *E. bovis* is rated second. In Europe *E. zurnii* is the most frequent cause of bovine coccidiosis. The acute disease is characterized by haemorrhagic diarrhoea, marked tenesmus, anaemia, weakness and emaciation. In severe infections death may occur as early as 7 days after the onset of clinical signs.

Major post mortem lesions occur in the large intestine, though general catarrhal enteritis may be present in both the small and large intestine. In severe cases the caecum and colon may be filled with semifluid hemorrhagic material. The epithelium may slough away leaving large denuded areas, which are infiltrated with lymphocytes and leukocytes. In less acute cases the mucous membrane is roughened and spotted with petechial hemorrhages. Large number of developmental stages of oocysts are seen in the smears from the mucosa (Abebe *et al*., 2008).

2.6. Factors Affecting the Pathogenicity of *Eimeria*

2.6.1 Host - Parasite relationship.

A successful parasite is one that infects every available host causing minimal damage. This ultimate, harmonious co-existence is however easily leading to produce disease in the host (Abebe *et al*., 2008).

2.6.2 Site of development.

The coccidia that invade the small intestine generally produce less pathogenic effect. This is because the calves have long small intestine providing a large number of host cells and allowing the potential for enormous parasite replication with minimal damage. If absorption is impaired, the large intestine is capable of compensating to some extent. In the large intestine the rate of cellular turnover is much less and there is no compensatory effect from other region of the gut. Thus produce intense pathologic condition (Gregory and Catchpole, 1990).

2.6.3. Effect of Age on Susceptibility to Infection

All ages of calves are susceptible to infection but younger ones are more susceptible to disease. During the first few months of life, the majority will probably have been infected and may or may not show signs of disease. According to Svensson *et al.* (1993) calves started shedding *Eimeria* oocysts as early as 2 weeks. However, in the young calves colostrum provides passive immunity during the first few weeks of life; thereafter they acquired resistance to coccidial infection as a result of active immunity (Hermosilla *et al*., 1999).

2.6.4. Other factors affecting pathogenicity

An animal resistance to coccidial infection can be lowered by adverse conditions such as dietary changes, prolonged travel, extremes of temperature, weather conditions, changes in environment or severe concurrent infection. Nutritional status, mineral and vitamin deficiencies can also influence resistance to infection (Gregory and Catchpole, 1990).

2.7. Clinical signs of bovine coccidiosis.

Calves are usually infected more among all age groups and show the clinical form. They appear unthrifty, perineum stained with feces, watery feces sometimes with blood (Coetzer and Justin, 2004; Fitzpatrick, 2006; Maas, 2007). Severely infected animals present with thin bloody diarrhea, which may persist for about one week, or merely thin feces with shreds of intestinal epithelium and mucus (Coetzer and Justin, 2004). Dehydration, weight loss, depression, anorexia, straining after defecation and occasionally death may occur (Kennedy, 2000; Maas, 2007). The patient may present

with mild fever in early stages though seldom. More often the temperature is normal or subnormal (Maas, 2007).

Coccidiosis is a self-limiting infections and spontaneous recovery occurs without specific treatment when the intestinal reproduction of parasite is completed (Coetzer and Justin, 2004; Fitzpatrick, 2006). Nervous sign may develop in some calves with acute intestinal coccidiosis (Kennedy, 2000). This condition is highly fatal (80-90%) within 24 hours of presentation of the first signs which include: muscle tremors, hyperesthesia, and clonic-tonic convulsions with ventral flexion of the head and neck and nystagmus (Radostits *et al*., 2000).

2.8. Epidemiology

Eimeria spp. are ubiquitous parasite that might present in all farms. A number of factors (environmental and host) influence the epidemiology of coccidiosis in cattle. These include, age of the animal, farm management, herd size and animal density. Management practices include population density, stocking in pasture, aeration, farm hygiene and sanitation (Rodriguez, 1996). Out of 24 reported *Eimeria* spp. only *E. bovis* and *E. zuernii* can cause clinical disease. All age groups are susceptible for infection whereas, calves under 1 months are mostly at risk. Most outbreaks occur following weaning (Radostites *et al*., 2000).

Adult cattle are found as the source of *Eimeria* oocyst for the calves and have been observed to shed low to high number of oocysts in several studies that act as the source of contamination for the calves while they are protected by the immunity following earlier exposure (Faber, 2002).

Continuous exposure to low numbers of oocysts from the adult animals results in endemic stability. Therefore, the mere presence of oocysts is not always related to clinical outbreak. However, clinical disease depends on the magnitude of the oocysts ingested. Calves with low oocyst exposure may not produce severe disease rather serves as immunity production for subsequent infection. But high infection pressure increases the individual risk to acquire clinical disease (Waruiru *et al*., 2000).

Poor hygiene in the calf rearing area gives a favorable condition for oocyst sporulation and survive longer in the environment. A low prevalence rate was seen with improved hygiene of calf pens (Chibunda *et al*., 1996).

Stress factors such as weaning, change in diet, climate condition, transportation, frequent regrouping, inadequate feeding or other infectious agents further contribute to infection. Stress due to harsh cold conditions was considered as one of the factor for winter coccidiosis in Canada (Radostites *et al*., 2000).

In North America *Eimeria bovis* is the most prevalent species. It mainly affects the colon and caecum resulting in severe enteritis and diarrhea in heavy infestations (Urquhart *et al*., 1996). The PPP (Peripatent period) ranges between 15 and 20 days and a patent period of 5 to 12 days depending on the infecting dose. During this phase it produces large oocysts that are egg shaped measuring 28 by 20 micrometers (Urquhart *et al*., 1996).

Many studies have been done in various parts of the world including Africa aimed at establishing the prevalence of this disease. In German dairy farms *E. bovis* and *E. zuernii* prevalence were found 76.9% and 83.1% respectively (Bangoura *et al.*, 2012). *E. zuernii* had a higher OPG (Oocysts per gram) (2,950) while *E. bovis* had OPG of 700, with the higher OPGs being recorded in calves (Bangoura *et al.*, 2012).

In Poland, Tomczuk *et al*., (2015) identified eight *Eimeria spp*. among calves where the prevalent species are *E. bovis* (37.4%), *E. zuernii* (19.9%) and *E. canadensis* (12.1%) with an overall 52.8% prevalence. Presence of *E. bovis* increases the occurrence of other *Eimeria spp.* also found in the same study.

In Turkey, out of 11 identified species *E. bovis* (28.5%) was the most prevalent species followed by *E. auburnensis* (17.2%) while *E. zuernii* came fourth (12.4%). The least prevalent was *E. bukidnonensis* (0.5%). Multiple infections were also confirmed in one host (Cicek *et al*., 2007).

Koutny *et al*., 2012 identified eleven *Eimeria* spp. from Austrian dairy farms including *E. bovis* (65.54%), *E. zuernii* (63.85%), *E. auburnensis* (56.76%) and *E. ellipsoidalis* (54.05%) having the highest prevalent.

From the study done in Estonian dairy farms Lassen *et al*. (2009) identified twelve *Eimeria spp*. by PCR and animals under 3-12 months was found commonly affected by Eimeria spp. where calves below 3 months shed most oocysts.

In a study carried out in central Kenya in dairy cattle, Waruiru *et al*. (2000) estimated the prevalence of coccidioisis at 30.9%. From this study, about eight species of Eimeria were identified. The most prevalent of these were *E. bovis* and *E. zuernii.* Season, age and farm had significant influence on the prevalence of *Eimeria*.

In Ethiopia, Dawid *et al*. (2012) reported that poor hygienic status of the farms and younger aged calves were strongly associated with infection of coccidiosis in dairy farms. However, in another study, Alemayehu *et al*. (2013) agreed that age was a significant factor but breed, body condition, sex, and management system were not significantly associated with the disease.

In a study done in South Africa Matjila and Penzhorn (2002) reported that the prevalence of coccidiosis was highest in the dairy farms in Pienaars River at 52%. There were various *Eimeria* species identified from the three localities. Most prevalent species in all three localities was *E. zuernii* and *E. bovis*.

Pfukenyi *et al*. (2007) carried a study in Zimbabwe aiming to assess the effects of geographical location, age, sex and season on gastrointestinal parasites, reported that age, pregnancy, lactation, high rainfall, and rainy season were significantly associated with high OPG counts of coccidia parasites in cattle.

In Pakistan at Toba-Tek Singh district, the prevalence of coccidiosis in bovines was estimated 47.09% (Rehman *et al*., 2011). *E. bovis* were identified as the most prevalent while other prevalent species are *E. zuernii, E. canadensis, E. ellipsoidalis, E. alabamensis* and *E. cylindrical*. However, prevalence was higher in the ground fed and pond watered calves compared to other management systems. This study concluded that occurrence of disease is influenced by the husbandry practices.

Similarly, a study was carried aiming to determine the prevalence and diversity of *Eimeria spp*. in Guwahati, Kamrup district, Assam, India. This study reported seven species of Eimeria with overall 11.9% prevalence in the study area. Infection is mostly prevalent in post wet season followed by wet, winter and pre-wet season (Das *et al*., 2015).

Samad *et al*. (2004) reported that 27% calves suffer from diarrhea is due to *Eimeria* spp. along with bacterial infection that brought in the Bangladesh Agricultural University Veterinary Clinic, Mymenshing.

In another study done in Vangura upazila in Pabna reported 4.11% *Eimeria spp*. infection among cattle population where females are more prone to gastrointestinal infection compared to males. Age and sex was found as significant factor for gastrointestinal parasitic infection (Islam *et al*., 2014).

Siddiki *et al*., (2009) carried out a study on Red Chittagong Cattle (RCC) aiming determination of prevalence of helminthiasis and protozoan agents. In this study 17% coccidial infection were recorded.

2.9. Immunity

Animals are protected by immunity following a primary infection and thus subsequent infections are generally not related to clinical disease (Svensson, 1996). Immunity is measured in terms of reduced pathogenic effect, decrease in the number of parasitic stages and improved body weight gain (Chapman, 1999).

Therefore, the degree of immunity depends on the initial exposure of oocyst. Larger doses (500 sporulated oocysts and above) always elicit better immunity (Daugschies *et al*., 1997). while low to moderate quantity of oocyst may not trigger the immune response sufficient to prevent subsequent infection and disease (Burger *et al.,* 1995). Protective immunity rapidly boosted by continuous exposure to oocysts (Hermosilla *et al*., 1999).

Maternal antibodies getting through colostrum protect calves during the early weeks of age from many diseases. Serum antibodies particularly IgM, IgA and IgG2 of cows have significant negative correlations between oocysts excretion and level of antibodies against *E. bovis* (Faber *et al.,* 2002). However, IgG2 serve as the major fraction in the humoral response to infection (Faber *et al*., 2002).

The immunity to *Eimeria* is very complex due to its different life cycle stages. Although several stage specific antigens are targeted by both humoral and cellular immunity, sexual stages are more susceptible to the immune response and provide a block in parasite maturation (Wallach, 1997).

E. bovis first generation schizont activate CD4+ TH1 cells. Although they can't cease the parasite life cycle possibly may interact with the level and duration of oocyst excretion in subsequent infection (Hermosilla *et al*., 1999).

Attempts to produce herd specific vaccine by radiation of oocysts isolated from feces induced only partial protection (Enemark *et al*., 2013). However, cattle remain exposed to infection throughout their entire life and moderate to low oocysts shedding may be observed even in adult cows and thus protection by immune system does not necessarily confer sterile immunity (Conrnelissen *et al*., 1995).

2.10. Diagnosis of *Eimeria* oocysts

In the case of hemorrhagic diarrhea containing tissue and fibrin always considered as coccidial infection. Microscopic examination along with qualitative fecal examination is the most commonly practiced procedure for *Eimeria* infection (El-Sawalhy AA, 1999).

Eimeria oocysts are easily identified with light microscopy using direct smear method on a microscope glass slide. The use of cover slip improves the optics and prevents soiling of the objective lens. Using saline solution instead of water is preferred as it prevents lysis of fragile trophozoites of protozoa (Dwight, 1995). However, sensitivity of this method reduced in diarrhoeic feces due to dilution and particularly in severe infection when large amount of blood, tissue or mucus are shed (El-Sawalhy AA, 1999). These include:

McMaster technique, a quantitative approach of analysis is highly reliable and simple to use (Levecke *et al.*, 2012). Thus it is commonly used in labs and analysis. It involves determining the number of nematode eggs and protozoan oocysts per gram of feces in order to estimate the level of infection in an animal. Using the principle of floatation, the oocysts are suspended in a fluid with higher density than water. The advantage of this method is that it is quick as the oocysts are floated free of debris before counting (Nolan *et al*., 2006).

Sedimentation technique is more appropriate for trematodes and acanthocephalan eggs and amoebas. Although it is more sensitive compared to direct smear but less sensitive than flotation for coccidian oocysts. In order to replicate similar success as flotation method, it is necessary to examine at least half of the sediment microscopically (Dwight, 1995).

Where more distinct conclusions are needed, the oocysts can be cultured to allow for sporulation and hence more specific identification (Dwight, 1995).

2.11. Economic Impact of Coccidiosis

Although it is quite impossible to estimate the accurate economic loss due to coccidiosis but it is considered sufficiently important economically in calves. Matjila and Penzhorn (2002) estimated that the lost profit amounts to $400 million/year. In a more recent study, the economic loss from coccidiosis is estimated at about $100 million each year (Mass, 2007). This huge amount of monetary loss is due to reduced feed efficiency, slower weight gain and increased susceptibility to other diseases, treatment cost and even death.

2.12. Treatment and control

The disease is self-limiting and clinical signs spontaneously subside when multiplication stage of the parasite passed. Prompt medication protect animal by inhibit development of stages, reducing the oocysts discharge and lessen the likelihood of the secondary infection (Radostits *et al*., 2000).

For treatment various anticoccidial drugs are used. These include: sulphaquinoxaline (3mg/kg/day for 3-5 days); amprolium (10mg/kg/day for 5 days); Monensin (2mg/kg/day for 20 days from the day of inoculation (Radostits *et al*., 2000; Mass, 2007). Sulphaquinoxaline is primarily act on the asexual stage of parasite and particularly useful for weaned calves that develop bloody diarrhea.

A single oral dose of 15 mg toltazuril /kg body weight (BW) and 1mg diclazuril/kg (BW) treatment prior to expected clinical disease efficiently controlled clinical disease in an artificial infection experimental study with *E. bovis* and *E. zuernii*. Diclazuril appeared to be more effective when compared with the control group. where the later has the higher efficacy compared to control group in terms of oocyst excretion, diarrhea score and average body weight gain (Philippe *et al*., 2014).

Coccidiosis has been difficult to control. The control of coccidiosis mainly depends on good hygiene practices, feeding from bunks, treatment of clinically infected animals and use of prophylactic anticoccidial drugs. As the oocysts are ubiquitous in nature it is difficult to treat the environment. Use of commercial disinfectants may reduce the infection pressure in animal house (Coetzer and Justin, 2004).

Chapter-3: Materials and Methods

3.1. Study area and duration

The study was carried out in selected areas under Chittagong district of Bangladesh. Its area is 5282.98 sq km, located in between 21°54' and 22°59' north latitudes and in between 91°17' and 92°13' east longitudes. It consists of 14 Upazilla (administrative locations) and Chittagong Metropolitan Area. This study was conducted in Chittagong Metropolitan Area and Patiya. 130 fecal samples from calves below 45 days old were collected from June, 2015 to December, 2015.

3.2. Development of questionnaire

A cross sectional survey design was followed to collect data from the selected farms. A questionnaire (for data collection) was formulated before initiation of the survey using closed ended (dichotomous and multiple choice) questions. A thorough literature review was done before formulating the questionnaire to gather information about the probable risk factor candidates for *Eimeria* spp. infection in calves. The questionnaire is attached as appendix.

3.3. Selection of study unit

This study area was carried out in Chittagong Metropolitan Area (CMA) and Patiya upazilla and study unit was individual calf. During farm selection within the study area it was emphasized to select and collect samples from different zones such as north, south, east and central zones of the study area. All calves under 45 days of age within the selected farms were selected for sample collection.

3.4. Sample collection

Samples were collected from calves as rectal swab. All calves aged less than 45 days with the history of diarrhea were sampled. Five grams of fecal samples was collected directly from rectum or immediately after defecation in a wide-mouth plastic bottle. Gloves were changed between calves to reduce the risk of contamination during sampling.

3.5. Transportation and preservation of sample

Thermo flask containing ice was used to transport the samples from the collection site to Clinical Pathology Laboratory, CVASU (Chittagong Veterinary and Animal Sciences University) for analysis. Samples were preserved in 10% buffered formalin and kept in the 4^0C until use (Zajac and Conboy, 2006). Proper labelling was done on collection bottles.

3.6. Parasitological examination

Fecal samples were analyzed using direct smear method for the presence of *Eimeria* oocyst. The procedure was adopted as described by Zajac and Conboy (2006). In brief, A small amount of feces was placed on the microscopic slide and a drop of water was added to the feces and mixed thoroughly followed by cover with a cover slip and then examine under microscope at 10X.

3.7. Data entry and statistical analysis

Field data obtained from the questionnaire and laboratory data were entered and collated into a spread sheet into spread sheet (MS Excel-2007 Program). All data were transferred to statistical software STATA version 12.1 (StataCorp LP, College Station, Texas, USA) to conduct the statistical analysis. Univariable logistic regression models were used to identify significant variables associated with the observed prevalence of *Eimeria* infection in the study area. Variables with p-value less than 0.2 in univariable analysis were selected for multivariable logistic regression analysis. A backward elimination procedure was followed to find out final regression model. In multivariable model, variables with p-value less than 0.05 were considered to be significant.

Chapter-4: Results

The study was carried out in selected farms of Chittagong Metropolitan area and Patiya upazila of Chittagong to know the prevalence of *Eimeria* spp. in the calves with the history of diarrhea. A total of 130 samples were collected from calves aged less than 45 days of 39 randomly selected farms. Both farm data and individual calf data were collected by administering standard questionnaire (Annex-I).

4.1. Prevalence of *Eimeria* in calves

Total 130 fecal samples of calves were examined in microscopy, 27 (20.77%) contained oocysts of *Eimeria* spp. Considering age prevalence of *Eimeria* in calves between 2-4 weeks and > 4 weeks were found 26% and 27%, respecitively. In case of closed barn type prevalence of *Eimeria* was found 31% while it was 20% in partially open type barn and 19% for open type barn (Table-1).

Farms where pond water was used as source of drinking water, prevalence of *Eimeria* was 38% while 19% and 10% prevalence were found in case of deep tube well and supply water respectively (Table-1).

Table-1 stated that no significant difference was found in prevalence of *Eimeria* considering sex, which was 22% in male and 19% in female. Almost no difference was found in prevalence of *Eimeria* when hygiene condition of the farm was tested as risk factor for the disease. In good scored farms, the prevalence was 20% while this was 21% in both fair and poor scored farms. Prevalence of *Eimeria* was found 20% (11-20 or > 50 animal/farm) and 22% (21-50 animal/farm) in different herd size (Table-1).

Prevalence of *Eimeria* was found more common in liquid fecal samples (37%) compared to solid (15%) and semi solid feces (18%). Similarly, in concrete type calving pen *Eimeria* prevalence was found 30% whereas this was 19% and 13% in case of brick and slatted floor. No significant difference was found for prevalence of *Eimeria* considering litter material like rubber pad (19%) with no litter used (21%). Prevalence of *Eimeria* was found quite high in mildly dehydrated (within 2 second) calves (75%) while it was found only 19% in severely (> 6 second) and moderately dehydrated (2-6 second) calves (Table-1).

Almost similar degree of prevalence (20% and 22%) was found in farms where bedding cleaning performed with or without disinfectant respectively. Prevalence of *Eimeria* was found 26% and 17% in calves separated from their dam > 24 hours and within 24 hours, respectively. In case of presence of other animals in farm, Prevalence of *Eimeria* was found 24% while 18% prevalence was found in farms with no coexistence of different animals.

4.2. Univariable logistic regression model

In univariable logistic regression analysis, several factors showed association with the presence of *Eimeria;* 2-4 weeks age (OR= 5.7) or >4 weeks age (OR=6.00) verses <2 weeks age; flooring in calving pen with brick (OR=1.48) or concrete (OR=2.81) versus slatted (OR=1); source of drinking water from deep tube well (OR=2.14) or pond (OR=5.4) versus supply (OR=1); liquid feces (OR=3.21) and semi solid feces (OR=1.20) versus solid (OR=1) feces; skin fold retention time within 2 second had more odds of getting *Eimeria* than time 2-6 sec or >6 sec (Table-1).

Table 1: Univariable analysis (logistic regression) to identify statistically significant variables influencing the occurrence of *Eimeria* in the study area

Variable	Level	Observation (N)	Number positive (%)	OR	P-value
Herd size	Small (11-20)	20	4 (20)	1	0.97
	Medium (21-50)	46	10 (22)	1.11	
	Large (>50)	64	13 (20)	1.01	
Age (week)	2	35	2 (6)	1	0.01
	>2 to 4	50	13 (26)	5.79	
	>4	45	12 (27)	6.00	
Sex	Female	72	14 (19)	1	0.67
	Male	58	13 (22)	1.19	
Type of barn	Open	73	14 (19)	1	0.57
	Partially open	41	8 (20)	1.02	
	Closed	16	5 (31)	1.91	
Flooring in calving pen	Slatted	52	7 (13)	1	0.11
	Brick	32	6 (19)	1.48	
	Concrete	46	14 (30)	2.81	
	Robber pad	36	7 (19)	1	0.81

Type of litter in calf pen	No litter	94	20 (21)	1.11	
Source of drinking water	Supply	10	1 (10)	1	0.16
	Deep tube well	104	20 (19)	2.14	
	Pond	16	6 (38)	5.4	
Fecal consistency	Solid	72	11 (15)	1	0.04
	Semi-solid	28	5 (18)	1.20	
	Liquid	30	11 (37)	3.21	
Dehydration test	Mild (Within 2 second)	53	14 (19)	1	0.05
	Moderate (Within 2-6 second)	73	10 (19)	0.98	
	Severe (>6 second)	4	3 (75)	12.64	
Parity of dam	1 to 2	49	9 (18)	1	0.81
	3 to 4	70	16 (23)	1.31	
	>4	11	2 (18)	0.98	
Hygiene score	Good	55	11 (20)	1	0.98
	Fair	56	12 (21)	1.09	
	Poor	19	4 (21)	1.06	
Bedding cleaning	Water cleaning	60	13 (22)	1	
	Water with disinfectant	70	14 (20)	1.10	0.81
Separation of calf from dam	Within 24 hours	54	9 (17)	1	0.22
	>24 hours	70	18 (26)	1.73	
Feeding of colostrums	Within 2 hours	115	25 (22)	1	0.59
	2 to 6 hours	13	2 (15)	0.65	
Presence of other animals in the farm	No	67	12 (18)	1	0.40
	Yes	63	15 (24)	1.43	

Table 2: Final model of multivariable analysis (logistic regression) to identify statistically significant variables influencing the occurrence of *Eimeria* in the study area

Variable	Level	OR	95% CI	P-value
Age (week)	2	1	-	
	>2 to 4	6.58	1.32-32.65	0.02
	>4	9.03	1.72-47.25	0.009
Fecal consistency	Solid	1	-	
	Semi-solid	1.45	0.43-4.84	0.54
	Liquid	4.54	1.53-13.44	0.006

4.3. Multilevel logistic regression model

To identify significant risk factors influencing the occurrence of *Eimeria* in the study area, a multilevel univariable logistic regression model was performed. In univariable logistic regression analysis, 5 variables (age, flooring of calving pen, source of drinking water, fecal consistency and dehydration test) showed significant (P-value <0.2) association with the outcome variable and therefore used in multivariable analysis. In multivariable analysis, the following two factors were found significantly (P-value <0.05) associated with presence of *Eimeria*: age and fecal consistency. Calves with more than 4 weeks of age and calves with 2 to 4 weeks of age had odds ratio of 9.03 and 6.58, respectively compared to calves with less than 2 weeks of age. Calves with liquid (OR=4.5) and semi-solid (OR=1.4) fecal consistency had higher chance to get the infection compared to calves with solid feces.

Chapter-5: Discussion

Neonatal calf diarrhea remains as common problem in livestock, causing great impact in terms of animal health and economic loss. Young stocks are at the greatest risk of diarrhea within the first month of life and the incidence of diarrhea declines with age (Daugschies and Najdrowski 2005). The aim of this study was to obtain up-to-date data on the prevalence and importance of *Eimeria* infections in study area and associated risk factors in neonatal calves. Particular strengths of this study included the diversity of farm sizes sampled, the study unit and diverse management practice within the sampling frame. We performed our study taking samples from operations with over 50 cows and operations with as few as 11 cows within the study area. Thus, our selected farms represent a general picture of the total population. This study used an approach to analyze the associated risk factors, which can be used in formulating target control program. We used advanced statistical analysis to identify risk factors responsible for the infection in calves in study area.

5.1. Prevalence of *Eimeria*

Although *Eimeria* is a common agent causing diarrhea in young animals (Rehman *et al*., 2011) no previous report about *Eimeria* prevalence and associated risk factors, is available to date in Chittagong, perhaps also true for the south east Bangladesh. This is the first time investigation of epidemiological aspects of *Eimeria* infection in calves. The prevalence of *Eimeria* was estimated 20.77% in this study. A similar result (19%, 20.76% and 20.04%)) was reported by Afzal (1996) in Paksitan; Priti *et al*. (2008) in India and Cicek *et al*. (2007) in Turkey, respectively. However, a higher prevalence (48%, 50%, 52%, 47.09%, 46% and 47.1%, respectively) was reported by Sanchez *et al*. (2008) in Argentina, Harpreet and Daljit (2008) in India; Matjila and Penzhorn (2002) in South Africa; Rehman *et al*. (2011) in Pakistan; Cornelissen *et al*. (1995) in Denmark; Dong *et al.* (2012) in China. On the contrary, in India a lower prevalence of 11.97% is documented by Das *et al*. (2015).

The differences in the prevalence from these previous studies may be due to variation in geography, management practices, housing system, climatic condition, study unit selection and hygienic measures. This study identified oocysts by direct smear method on the other hand Sanchez *et al*. (2008), Harpreet and Daljit (2008); Matjila and Penzhorn (2002); Rehman *et al*. (2011) used the floatation and McMaster technique for

the oocysts determination and OPG count. Differences between results suggest higher sensitivity of floatation technique than direct smear. In previous studies Rehman *et al*. (2011); Cornelissen *et al*. (1995); Dong *et al*. (2012); Chibunda *et al*. (1997); took samples from all ages that gave a higher prevalence while we obtained only calves samples for our investigation. Therefore, getting lower prevalence due to lower sample number in this study can't be denied. However, as calves are more prone to this infection compared to adult, it might be more authentic to collect samples from risk groups.

5.2. Risk factors associated with prevalence of *Eimeria*

Multivariate logistic regression model enabled us to identify the significant risk factors associated with *Eimeria* infection in calves. Age and fecal consistency were found significantly associated with the occurrence of *Eimeria*. Calf aged between >2 to 4-weeks or >4 weeks versus < 2-week was found significantly associated with the prevalence of *Eimeria.* This finding is in agreement with Daugschies and Najdrowski (2005); Lentze *et al*., (1999). They reported that the problem is more common in young animals from 3 weeks to 3 months of age. Thus early weeks of life is the main age for occurrence while very early age is protected probably due to maternal antibody. In our findings watery feces was significantly associated with *Eimeria* which is in accordance with those previously recorded by other investigators Daugschies and Najdrowski (2005); Lassen *et al*. (2009); Rehman *et al*. (2011). Although, not statistically significant slatted floor decreases the risk of Eimeria compared to brick and concrete type. This result is in line with the findings of Bangoura *et al.* (2011) and Daugschies *et al*. (1997). They reported having a slatted floor reduced the risk of presence of oocysts of pathogenic *Eimeria* spp. on the farm. Bangoura *et al.* (2011) mentioned the importance of cleaning and disinfection but we didn't observe a lower prevalence of *Eimeria* spp. if hygienic measurements like cleaning was practiced. It was evident that calves in farms, which received pond water as drinking water source were more susceptible to *Eimeria* (38%). Rehman *et al*. (2011) also found the similar results. Reason for higher prevalence of Eimeria in pond-watered animals may be due to contamination of water in ponds that concentrates the hosts and parasites within a restricted area.

Chapter-6: Conclusion

The intent of this study was to describe the *Eimeria* spp. infections in neonatal calves. Particular strengths of this study included the large number of herds and the diversity of farm sizes sampled. Multivariable logistic regression model was framed to find the risk factors behind the prevalence of *Eimeria* spp. The prevalence of *Eimeria* spp. was 20.77% in neonatal calves below 45 days of age. Age, flooring of calving pen, source of drinking water, fecal consistency and dehydration test were found significantly associated with prevalence of calf diarrhea caused by *Eimeria* spp. The results of the present study identify the risk factors associated with the spreading of *Eimeria* causing diarrhea will be helpful for developing suitable target control program in study area.

Chapter-7: Recommendation

This study was done for finding the farm and animal level factors associated with calf diarrhea in Chittagong Metropolitan area and Patyia. There are remarkable other etiological agents who can cause neonatal calf diarrhea in correlation with or without *Eimeria* spp. The OPG count was not done in this study. Adult animal samples were not analyzed to estimate the farm load of *Eimeria.* Species differentiation was also not determined. Based on above limitations future approaches can be as below:

a. Calves should be provided supply water as source of drinking water.

b. Slatted floor is recommended in calving pen.

c. Calves > 2 weeks of age should rear with care to avoid infection.

d. Species differentiation based on morphology and molecular detection can be done.

e. Molecular identification and prevalence of other etiological agents (*E. coli*, *Salmonella* spp. rotavirus, coronavirus, *Campylobacter jejuni*, *Cryptosporidium* spp. etc.) that can cause neonatal calf diarrhea in Chittagong.

f. Examination of detail postmortem changes need to be performed in case of dead animal.

g. Developing control model based on identified risk factors.

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Annex-I

Questionnaire for Factors associated to *Eimeria spp.* in calf diarrhea

1. Serial no. : ……. Date:…….
2. Name of the farm and owner: ……
3. Educational Status……..
4. Upazilla/Thana: ……...District: ……..…
5. Location: ……..Latitude: ……Longitude: ……..
6. Region of location: ☐Plain☐Hilly☐Coastal
7. Herd size:☐5-10☐11-20☐21-50☐>50 (Numbers……..)
8. Number of Calves:……
9. Population density: no.of animals..../sft
10. Age:
11. Sex: ☐Male☐Female
12. Breed of dam: ☐Local☐cross
13. Type of barn: ☐Closed☐Partialy open☐Open barn
14. Flooring type in calving area: ☐Concrete☐Sletted☐Brick☐Grass
15. Type of litter in calf pen: ☐Straw☐ Rubber pad ☐Litter less
16. Source of drinking water: ☐Ponds☐River☐Deep tubewell☐Supply☐More than one type
17. Bedding cleaning method: ☐Water cleaning ☐water cleaning with disinfectant
18. Calving month:☐J☐F☐M ☐ A ☐ M ☐J☐ J ☐ A ☐ S ☐ O ☐N☐D
19. Separation of calf from dam: ☐Immediately☐<24 hr☐>24hr☐No information
20. First feeding of colostrum after birth: ☐Within 30 min☐within 2 hr☐within 2-6hr☐no information
21. Feeding calf with waste milk: ☐Yes☐No (☐from mastitis☐Contain antibiotics)
22. Sucking as feeding regimen: ☐Yes☐No (☐Restricted☐No restricted)
23. Confinement from birth: ☐Single☐Group
24. Maximum age difference between youngest and adult calf housed in same pen: ☐<4wk☐4-8wk☐>8 wk
25. Diarrhoeic calves in farm: ☐Yes☐No
26. History of calf scour:☐Yes and dead☐Still Diarrhoeic☐Recovered☐ No
27. Therapy:☐ Antibiotics ☐ Antiparasitics ☐ Others ☐None
28. Feces consistency:☐ Liquid☐Semi-liquidor semi-solid ☐Solid or formed
29. Dehydration (Skin fold test): ☐Within 2 second☐Within 2-6 second ☐> 6 sec
30. Body weight(kg):
31. Newly introduced calves from other farms within----days/months:☐ Yes☐No
32. Dystocia during delivery of this calf:☐Yes☐No
33. Parity: ☐1st☐2nd☐3rd☐4th☐5$^{th/}$ ☐ More
34. Hygiene of calf feeding utensils:☐Not shared☐Shared & rinsed with water☐ Shared and disinfected
35. Physical contact with other: ☐None☐ unweaned ☐ weaned ☐> 6 month ☐adult

36. Surrounding environment of the farm:☐ Good ☐ Moderate☐Bad
37. Drainaige system: ☐ Good ☐ Moderate☐Bad
38. Number of other animals on the farm and if any have diarrhoea: Yes/No
    Bull: Sheep: Goat: Poultry:
39. History of calf death within -----days/months: Yes ☐ No; If yes, mention number:
    Clinical signs: ☐ Respiratory ☐ Digestive ☐ Still birth
40. Floor disinfection system: ☐ Yes ☐NoIf yes, Frequency: ------/month; Name of agent using now:
41. Grazing system: ☐Zero☐Community grazing☐Tethering; If zero grazing, Practice of washing before offering:
    ☐ Yes ☐ No
42. Storage system of feed: ☐ Good ☐ Fair ☐Poor
43. Is calf experiencing concurrrent condition: ☐ Respiratory ☐ Umbilical ☐ Others……..
44. If diarrhea calves present, any of your family members has loose motion? ☐ Yes ☐ No

Signature of Interviewer

Annex-II

Definition for grading qualitative variables

1. Surrounding environment

Good: Clearly separated from household, away from road, and clean.

Moderate: Near to household but away from road, and clean.

Poor: Near to household, road and unclean.

2. Drainage system

Good: Clearly circulated all the time and cleaned at least twice/week.

Moderate: Clearly circulated only at the time of washing and cleaned at least once/week.

Poor: Stagnant of wastage all the time and irregular cleaning.

3. Food storage

Good: Separated from the farm as well as good light and ventilation in storage room.

Moderate: Adjacent to the farm as well as good light and ventilation in storage room.

Poor: Adjacent to the farm as well as poor light and air circulation/ don't have any separate storage room.

4. Hygiene Score

Good: Farms having at least 2 good score without any poor for above 3, pre and post dipping during milking, disinfection of floor at least once/week.

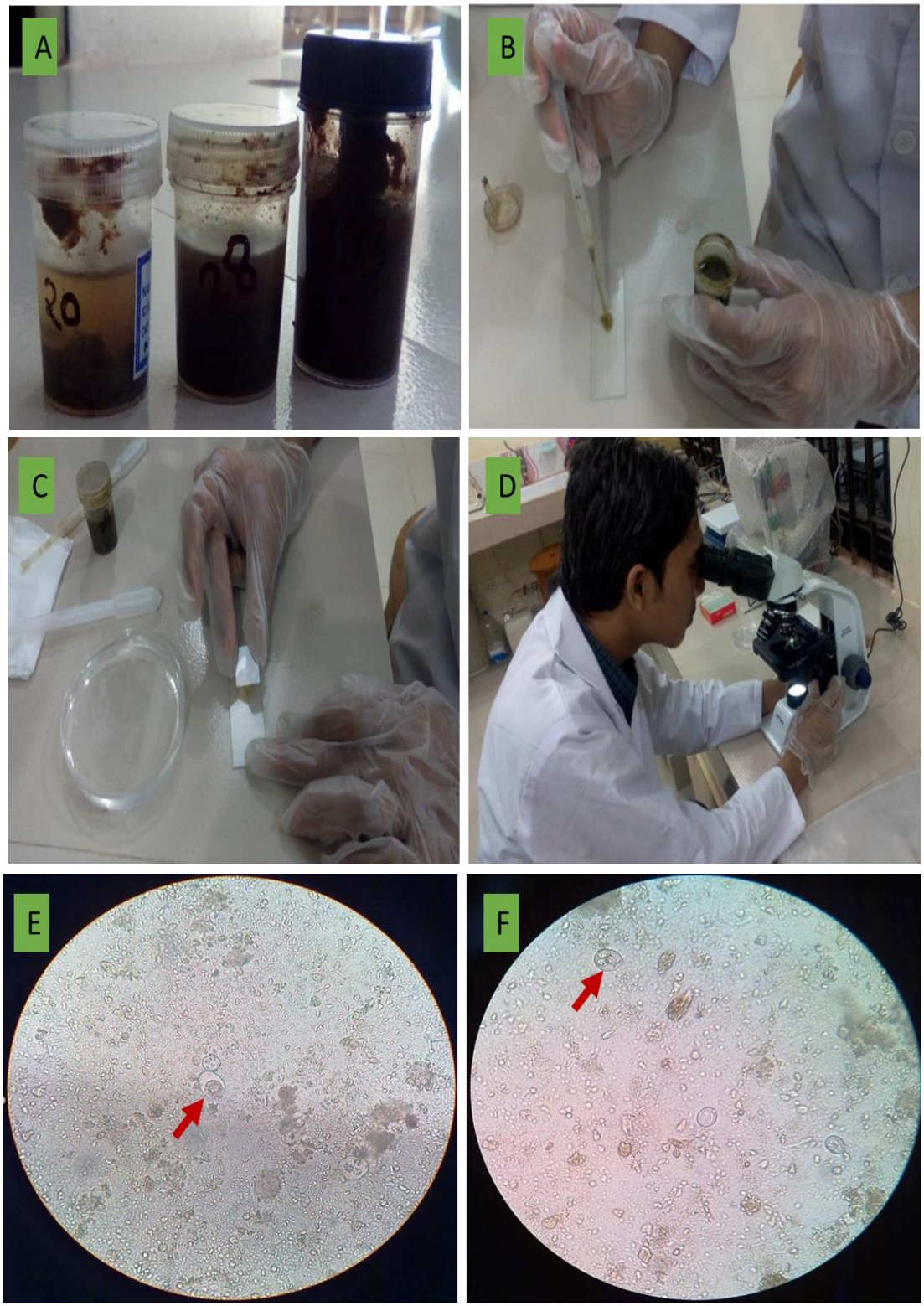
Moderate: Farms with at least 1 good score for above 3, pre/post dipping during milking, disinfection of floor at least once/15 days.

Fair: Farms with at least 2 moderate/fair marks for above 3, no dipping during milking, disinfection of floor at least once/month.

Poor: Farms with score below than above, no dipping, water cleaning only / no disinfection practice.

Annex-III

Activities during the research work



A: samples in container, B: Fecal sample place on slide, C: Place cover slip,

D: Observation of oocysts, E: Unsporulat ed oocyst, F: Sporulated oocyst.

(G-J): Observation of different farm factors for risk analysis

Brief Biography of the Student

This is Mohammad Rafiul Hoque passed the Secondary School Certificate Examination in 2006 followed by Higher Secondary Certificate Examination in 2008. He obtained his Doctor of Veterinary Medicine Degree in 2013 (held in 2015) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh with CGPA 3.59 (out of 4.00). Now, he is a candidate for the degree of MS in Pathology under the Department of Pathology and Parasitology, Faculty of Veterinary Medicine, CVASU. He published 1 scientific articles in international journal. He has immense interest to work on infectious diseases of livestock and their molecular pathobiology in the host.