CLINICO-PATHOLOGICAL INVESTIGATION OF CHICKEN COCCIDIOSIS AT DIFFERENT UPAZILA IN BOGURA DISTRICT

A THESIS

BY

MD. MOFIZUL ISLAM

Registration No. 1705440

Semester: July to December, 2018

Session: 2017-2018

MASTER OF SCIENCE (M.S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2018

Dedicated

To My

Beloved Parents

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ABSTRACT

The study was conducted to investigate the clinico-pathological status of chicken coccidiosis in the small scale commercial farms at different upazilla like Sadar, Gabtali, Sariakandi, Sherpur, Shajahanpur, Shibganj, Adamdigi, Dhunat, Sonatala, Dhupchachia in Bogura district from July to December, 2018. A through clinical and necropsy examination was done to record characteristics clinical signs and gross lesions. Different organs mainly small intestine and caecum were collected, preserved and processed for histopathological examination. A total of 343 diseased and dead chickens were examined out of 52 (20.6% in broiler, 10.47% in sonali, 10.25% in layer) chickens were found to be positive for chicken coccidiosis. The proportional mortality rates of coccidiosis were 19.25%, 21.42%, 8.23%, 7.5% respectively in age group of 0-4 weeks, 5-6 weeks, 7-8 weeks and above 8 weeks. The mortality rate was highest in 5-6 weeks age group (21.42%) and lowest in above 8 weeks age group (7.5%). The clinical signs of affected chickens were depression, ruffled feather, bloody diarrhoea, anaemia, drooping wings, reduction of feed and water intake. At necropsy deeper layers contained large areas of congestion and pinkish or blood tinged catarrhal exudates. Histopathologically, destruction of normal architecture of caecum and proliferation of fibrous connective tissue of liver. The villi of the mucosa were destroyed and disorganized and there was no continuation in the lining epithelial cells of villi.

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CHAPTER I

INTRODUCTION

In Bangladesh, Poultry industry plays an important role in the rural socio-economic system by contributing significantly on economic growth and simultaneously creating numerous employment opportunities. Chicken meat is also relatively cheap and affordable source of animal protein. Poultry production is an easy and efficient way of producing animal protein. With less capital investment relatively more profit could be earned by producing poultry. The poultry population of Bangladesh has increased from around 71 million in 1986 to around 188 million in 2006, an increase of about 164 percent in 20 years (FAO, 2008; BBS, 2017). The economy of Bangladesh is agro-based. Agriculture generated 14.10% of the GDP which the contribution of livestock sub sector comes about 1.54 % (Bangladesh Economic Review, 2018). In the rural area of Bangladesh more than 65% family rears poultry and the poultry population in Bangladesh is only 215.00 million (BBS, 2017).

A total of 5 million people are engaged in this sector (Saleque, 2007). At present chicken contributes 51% of total meat production in Bangladesh (Raha, 2007). Traditionally in Bangladesh, poultry rearing is one of the most important sources of income for rural women especially for landless and marginal farmers (Badruzzaman *et al*., 2015). There are about 110,800 small and large scale poultry farms in this country (Anon, 2006) and per capita annual consumption of meat is 5.99 kg against the universal standard 80 kg per head (Raha, 2007). Chicken is one of the important sources of animal protein (Candra,2013).There are several constraints of poultry industries in Bangladesh including outbreak of infectious diseases causing economic loss and discouraging poultry rearing (Das *et al.*, 2005). Various feed additives are given to chicken to increase growth in a very short time. However, several diseases cause severe reduction in the rate of growth in chicken. One of this disease is coccidiosis responsible for immunosuppression in the host (Candra, 2013). Coccidial infection brings a great loss to poultry industry.

Poultry industry is an emerging agribusiness starting practically during eighties in Bangladesh. But mortality and morbidity of chicken due to various infectious and non-infectious diseases is a major constrain for profitable poultry production. Coccidiosis is

one of the major constrain for the development of poultry in Bangladesh. The economic significance of coccidiosis is attributed to decrease production with higher feed conversion, growth depression and increased mortality and the cost involved in treatment and prevention, (Peek & Landman, 2011). Coccidiosis cannot be controlled by vaccination due to mixed infestation of more than one species of *Eimeria*.

Coccidiosis is a protozoal disease in poultry. *Eimeria spp*. is belonging to the phylum Apicomplexa causing coccidiosis of birds. *Eimeria tenella* is the most important species, as it causes caecal coccidiosis in chickens (Shirley *et al*., 1986). Intestinal coccidiosis caused by various species of *Eimeria*, is an economically important (estimated to be 2 billion dollars a year) disease of poultry (Zhang and Zeng, 2005). *Eimeria tenella* sporozoites invade the intestinal epithelium at a highly specific site in the linings of caeca of exposed chickens (Vervelde and Vermeulen *et al*., 1993). The mortality of young birds is predominant features. It is well known that poultry diseases are the major constraints for the developing the poultry industry (Karim, 2003). Poultry farmers face a wide range of diseases, which reduce the optimal productivity of poultry farms. On an average, 30 % poultry birds die annually in Bangladesh due to outbreak of several diseases (Ahmed and Hamid, 1992). Bogura is the northwest part of Bangladesh with different geo-climatic condition. Poultry diseases are the major constraints for developing the poultry industry in the Bogura region. Prevalence of a disease depends upon various factors such as geo-climatic condition, management practice and vaccination etc. The true picture as to incidence and pathology of coccidiosis in chicken has not been worked out yet in this country. Until some basic information regarding this disease problem are available, it is very difficult to encourage commercial farming in the country.

Keeping these views in mind, the present study has been under taken with the following objectives:

i. To determine the prevalence of chicken coccidiosis in relation to age group and variety of chicken
ii. To observe the clinical feature of chicken coccidiosis at different farm of Bogura district
iii. To observe the gross and histopathological changes of different organs developed due to chicken coccidiosis

CHAPTER II

REVIEW OF LITERATURE

Available literature for the determination of Pathology of chicken coccidiosis in small scale commercial farm is reviewed in this part of the thesis after a brief overview on the history, epidemiology, etiology, pathogenesis and pathology (gross and microscopic lesions), clinical manifestations, life cycle and economic importance against chicken coccidiosis.

2.1. Coccidiosis

Coccidiosis is a self-limiting, major infectious parasitic disease affecting mainly the intestinal tract of poultry and is caused by the Apicomplexan protozoan of the genus *Eimeria*. The condition most commonly occurs under intensive rearing conditions, where pathogenic populations of the causative agent may build up. Chicken coccidiosis is an enteric parasitic disease caused by multiple species of the protozoan parasite of the genus *Eimeria* and is one of the commonest and economically most important diseases of poultry world-wide; causing production losses, high morbidity (due to acute bloody enteritis) and mortality rates (Shiny *et al*., 2005).

2.2. History of Coccidiosis

Coccidia possess a somewhat complicated history in the story of how they came to be a part of the taxonomic classification of which they are currently recognized. The first coccidia were observed by Leeuwenhoek in the late 17th century and consisted of oocysts that were found in rabbit (Levine, 1982). As a whole, the genus known as *Eimeria* is the largest of the *Eimeriidae* family and belongs to the phylum Apicomplexa of the subkingdom Protozoa which is characterized by the presence of an apical complex in the sporozoite stage of the parasite. All apicomplexans are characterized as intracellular parasites (Levine, 1982; McDougald and Fitz-Coy, 2008). Members of the genus, *Eimeria*, are classified as having oocysts with four sporocysts, each with two sporozoites, and are considered homoxenous, meaning that all endogenous stages occur within a single host. Of this genus there are approximately 1200 named species, capable of infecting and causing disease in a wide range of host organisms (Current *et al*., 1990). Coccidia of this genus are primarily host specific with certain species infecting only a

single host species or a group of closely associated hosts (Conway and McKenzie, 2007). Originally, the disease in chickens was believed to be caused by a single species, *Eimeria avium* (Edgar, 1958). However, research performed by Tyzzer (1929) elucidated the fact that multiple species of *Eimeria* were capable of causing the disease in chickens as well as in other species. There are currently nine species of *Eimeria* known to parasitize chickens: *Eimeria acervulina*, E. brunetti, *E. maxima, E. mitis, E. mivati, E. necatrix, E. praecox, E. hagani,* and *E. tenella* (McDougald and Fitz-Coy, 2008)

2.3. Oetiology

2.3.1. Classification of Coccidiosis

Kingdom: Protista

Phylum: Apicomplexa

Class: Conoidasida

Order: Eucoccidiorid

Family: Eimeriidae

Genus: *Eimeria*

Species: *Eimeria tenella, Eimeria maxima, Eimeria necatrix, Eimeria acervulina, Eimeria brunetti* and *Eimeria mitis.*

2.3.2. Most Common Species

Most coccidia in poultry belong to the genus *Eimeria*, which are highly host specific. Seven species of *Eimeria* are widely recognized as the causative agents of coccidiosis in chickens, of which *E. tenella, E. necatrix*, *E. maxima* and *E. brunetti* are highly pathogenic, *E. acervulina* and *E. mitis* are less pathogenic, while *E. praecox* is regarded as the least pathogenic (McDougald, 2003;). Most common spesies in Bangladesh *Eimeria tenella, Eimeria maxima, Eimeria necatrix, Eimeria acervulina* and *Eimeria brunetti* are the cause of coccidiosis in poultry. Among them the occurence of *Eimeria acervulina* and *Eimeria brunetti* in poultry in Bangladesh is reported for the first time (Karim *et al*., 1990). The following species of *Eimeria* comnonly occur in chicken in Great Britain. These are *E. tenella, E. maxima and E. immities* by the means of

identification of coccidial oocyst in deep litter in poultry house (Davies *et al*., 1955). A brief practical account of coccidiosis as it occurs among chicken reared on litter. The following species of coccidia occurs commonly: These are *E. tenella, E. acervulina, E. brunetti, E. maxima, and E. mivati. E. necatrix* and also are found some times *E. hogani, E. mitis* and *E. praecox* (Reid *et al*., 1977). The causative organisms are identified and classified by their morphological and behavioral characteristics. The large numbers of oocytes produced by infected chickens are sufficiently distinct for them to be used as a means of classification by microscopic examination. Although coccidial infections can be confirmed by the presence of oocysts in the faeces, the presence of these can have little or no relationship to an impending or existing infection (Joyner, 1978).

2.3.3. Most Pathogenic Species

E. necatrix and *E. tenella* are the most pathogenic in chickens. Infection with *E. tenella* can be recognized by blood in droppings and faeces around the cloaca. Other important less pathogenic strains affecting chickens include *E. acervulina, E. maxima, E. praecox* and *E. mitis, E. adenoides* and *E. meleagrimitis* are the most important causes of the disease (Tyzzer, 1929; Levine, 1983). The pre-eminently pathogenic species of coccidiosis are *Eimeria tenella* which attacks the caecal wall and produce an acute hemorrhagic type of disease. *E. necatrix* which attacks small intestine to produce an acute initial attack resulting in early death or a lingering illness characterized by progressive emaciation and general unthrifliness. He stated that *Eimeria maxima* is far less lethal than *Eimeria tenella* and *Eimeria necatrix* (Becker, 1959). The intestinal surface damage caused by different species of *Eimeria* and reported that *Eimeria brunetti* caused the most severe mucosal damage when compared *Eimeria mivati, Eimeria necatrix* and *Eimeria maxima* (Witlook *et al.,* 1977).

2.4. Epidemiology

2.4.1. Geographical distribution and prevalence of Coccidiosis

Coccidiosis is worldwide distributed (Macpherson, 1978). The prevalence of coccidia in Bangladesh Agricultural University Poultry Farm is 54.14%, among them 23.75 percent was *E. tenella*, the most prevalent one (Mondal *et al.,* 1978). The proportion incidence of coccidiosis is lower and it is 8.71%, because the farmers are intensely aware of coccidiosis and other parasitic disease now a days. In West Bengal 85 (10.91%) cases of

coccidiosis is recorded (Bhattacharya Pramanik, 1987). Drug resistance to anticoccidial drugs is described worldwide to all coccidiostats and to all *Eimeria* species (Zhang *et al.*, 2013).

2.4.2. Seasons

Although Coccidiosis generally occurs round the year but more frequently occurs during the warmer months of the year (Smith, 1995). Coccidiosis generally occurs more frequently during warmer (May to September) than colder months (October to April) of the year (http://www.uniprot.org, 2009).

2.4.3. Susceptible Hosts

Coccidiosis is a parasitic disease that affects the poultry. Seven species of *Eimeria* are known to infect chickens and they show a wide variation in their pathogenicity. In addition, two further species have been described, namely *E. hagani* and *E. mivati*, but further studies on the importance of these species are needed (Conway and Mckenzie, 2007). In turkeys seven species of *Eimeria* have been reported, however *E. innocua* and *E. subrotunda* are considered non-pathogenic (Trees, 1990; McDougald, 2003). Geese are parasitized by two species; *Eimeria truncata* (unusually this is found in the kidney) and *Einieria anseris*. A large number of specific coccidia have been also reported. The most pathogenic coccidial infection of ducks is *Tyzzeria perniciosa*, which causes hemorrhagic enteritis in ducklings less than 7 weeks of age (Trees, 1990; McDougald. 2003). Coccidiosis rarely occurs in layers and breeders, although in situations where there is an immunity breakdown all pathogenic *Eimeria* species may cause an abrupt and severe drop in egg production for three to six weeks. Any recovery from severe infection can take 10-14 days, and it takes longer to production status (Williams, 1996).

2.4.4. Susceptible Age

Young chickens are more susceptible and more readily display signs of disease, whereas older chickens are relatively resistant as a result of prior infection. Typically, the disease is seen in chickens of 3-6 weeks old, before they have acquired immunity. Chickens are commonly attacked by coccidiosis and heavy mortality occurred among the 2-4weeks old birds (Kamath, 1955). The excystation of *E. tenella* sporozoites more rapid in chicks aged 4, 5 and 6 weeks than in those 0, 1, 2 and 3 weeks of age. Also in birds 0-1 weeks of age, a greater proportion of sporulated oocysts are discharged in the feces a few hours

after inoculation (Rose, 1999). Day 5 as the most severe stage of infection to histological and ultra- structural changes and decrease in nutrient absorption (Humphrey, 1973). Two week old chickens are susceptible to *E. acervulina* (duodenum), *E. maxima* (jejunum), *E. brunetti* (ileum) and *E. tenella* (caecum) resulting weight loss, intestinal lesion scores (Kogut *et al*., 2005). Chickens are commonly attacked by coccidiosis and heavy mortality occurred among the 2-4weeks old birds (Kamath, 1987).

2.4.5. Site of Infection

The various stages of the parasite are distributed throughout the mucosa of the posterior half of the small intestine, rectum, caeca and cloaca and also the upper portion of the small intestine in heavy infection due to *E. brunette* (Levine, 1942). *Eimeria tenella* attack the caecal wall and produce an acute hemorrhagic type of disease. *E. necatrix* which attacks the small intestine to produce an acute initial attack. *Eimeria brunetti* which distributes itself in the mucosa of the lower half of the small intestine, rectum and cloaca, causing more or less continuous light daily losses of the flock but leaving the chickens in normal flesh, *Eimeria maxima* attacks the middle and lower small intestine. *Eimeria accervulina* attacks mucosal layers of the villi and the sporozoites enters and migrates to the epithelial cell lining, the gland and fundus via macrophage (Becker. 1959). The intestinal surface damage caused by different species of *Eimeria* are complete villar destruction, caecal core formation through the villus tip in the jujenum, damage to the mucosal surface, epithelial sloughing and isolated patches of exposed connective tissue in the jujenum. *Eimeria mivati* damaged the villus tip of the duodenum and caused sloughing of the villiar epithelia exposing the lamine propria (Witlook and Ruff, 1977).

2.4.6. Mode of Transmission

Coccidiosis has been shown to be common to intensively managed commercial poultry farms especially where management or hygienic standards are compromised (Adene and Oluleye, 2004). Fly (*Musca domestica*) can spread the oocyst of coccidia over a wide area. *Eimeria acervulina* may parasitize the caeca when large number of sporozoites is directly introduced into the caeca. Both schizont and gametocyte develops by parasitization of the caeca was never heavy (Joyner and Norton, 1971). The oocysts are extraordinary resistant to environmental stress and disinfectants, remaining viable in the litter for many months. Temperatures above 56°C and below 0°C are lethal but it seems to be impossible to decontaminate a previously contaminated poultry house or

enviroment. Sporulated oocysts can be spread mechanically by wild birds, insects or rodents and via contaminated boots, clothing, equipment or dust. Direct oral transmission is the natural route of infection (McDougald, 2003).

2.4.7. Morbidity and Mortality Rates

The mortality rate due to caecal coccidiosis is the highest among coccidiosis (Seneviranta, 1969). Morbidity could be variable and mortality could reach up to 58.2% in field outbreaks (Norcross and Washko, 1970). Coccidiosis was found in 58.2% of the cases. It is concluded that since a diagnosis of coccidiosis is histologically confirmed in only 58.2% of the cases of coccidiosis diagnosed clinically, this is a poor criterion by which to assess drug resistance. The mortality of poultry chickens at the Bangladesh Agricultural University Poultry Farm 14.66% due to coccidiosis (Kutubuddin, 1973). Coccidiosis was the cause of death in 38 (15.8/6) percent chickens at Panjab Agricultural University, India (Sen *et al*., 1981).

2.4.8. Risk Factors

The severity of an infection depends on; the age of chickens, *Eimeria* species, number of sporulated oocysts ingested, immune status of the flock and environmental management. Chickens reared on litter are always at risk. High stocking rates and the resulting environmental conditions are important factors. Warm, wet and under- ventilated conditions are ideal for massive multiplication. When chickens are in direct contact with their droppings, then the risk of infection is greatly increased. Oocysts may remain in buildings from a previous batch of birds, and they may be carried by mechanical means, including equipment, clothing, insects and other animals. Chickens introduced to an infected building will quickly become infected. Examined risk factors on chicken's farms and found that poor hygiene related to personnel, feeding and drinking was important, as were the presence of other diseases on the farm and *Eimeria* species found in the previous flock (Graat *et al*., 1998). Whole wheat feeding, compared with a complete ground and pelleted feed, has been shown to increase parasite development during infection with the *E. tenella*. This might be explained by modifications of digestive physiology and intestinal microflora by whole wheat (Gabriel *et al*., 2003). Coccidiosis are involved in primary or secondary disease in 35 percent cases in fowl. (Poal, 1969). Chickens mortality occurs in coccidiosis reveals four major physiological stresses before death: (1) Hypothermia (2) Depletion of carbohydratestores (3) Metabolic

acidosis and (4) Renal tubule cell dysfunction. These stresses were pronounced in chicks surviving the infection (Witlock *et al*., 1981). The contents of amylopectin granules in freshly exeysted sporozoites of various species of *Eimeria* and found *Eimeria acervulina* and *Eimeria haganii* which paracitize favourably in the upper part of the small intestine of chicken contained very small amount of amylopectin and *E.maxima* which parasitizes in the middle part of the small intestine contained a small amount of amylopectin. *Eimeria tenella* which parasitezes in the caecum contained a large amount of amylopectin (Nakai *et al*., 1981). The course and clinical appearance of an *Eimeria* species infection in chicken flocks depend on the response of an individual chicken to infection and on population dynamics of the infection in the flock. Differences in ingested numbers of oocysts may affect oocyst load in the flock and the subsequent infectious dose for not yet infected chickens. To study the link between numbers of oocysts excreted by infected chickens and transmission of *Eimeria acervulina*, experiments were carried out with 42 pairs of chickens using inoculation doses with 5, 50, 500 or 50,000 sporulated oocysts. In each pair one chicken was inoculated and the other chicken was contact exposed. All contact chickens became infected, which occurred on average within 34 hour after exposure to an inoculated chicken. Although a higher inoculation dose resulted in higher oocyst excretion in inoculated and contact infected chickens, only small non-significant differences in transmission rates between groups were found (Velkers *et al.,* 2010).

2.5 Life Cycle

Fantham (1910) was the first to describe the entire life cycle of an *Eimeria* parasite in a chicken host. Later on, Tyzzer (1929) published detailed descriptions of the life cycle stages of various *Eimeria* spp. (*E. acervulina, E. mitis, E. maxima* and *E. tenella*) in sections of intestines. In 1932, he also described details of the life cycle of *E. praecox* and *E. necatrix* (*Tyzzer et al*., 1932).

More recently, the life cycle of chicken *Eimeria* spp. has been well documented by various other authors (Long & Reid, 1982; Fernando, 1990; McDougald, 2003). Although numerous drawings on the life cycle of chicken *Eimeria* spp. have been published, the website of the book "Encyclopedic Reference of Parasitology by Heinz Mehlhorn" (2001).

Table 1: Oocyst habitat, morphology and pathogenicity of *Eimeria* spp. in farm birds (Levine, 1985)

Species Chickens	Host/Habitat	Oocyst size (µm)		Shape	Pathogenicity	Reference
		Length	Width			
E. acervulina	Small intestine	12-23	9-17	Ovoid	Low	Tyzzer, 1929
E. brunetti	Small intestine, rectum, caeca, cloaca	14-34	12-26	Ovoid	Moderate	Levine, 1942
E. hagani	Small intestine	16-21	14-19	Ovoid	Low	Levine, 1942
E. maxima	Small intestine	21-42	16-30	Ovoid	Low to moderate	Tyzzer, 1929
E. mitis	Small intestine	10-21	9-18	Subspheric	Low	Tyzzer, 1929
E. mivati	Small, large intestine	11-20	12-17	Ellipsoid or ovoid	Low to moderate	Edgar & Siebold, 1964
E. necatrix	Small intestine, caeca	12-29	11-24	Ovoid	High	Johnson, 1930
E. praecox	Small intestine	20-25	16-20	Ovoid	No	Johnson, 1930
E. tenella	Caeca	14-31	9-25	Ovoid	High	Raillet & Lucet, 1891

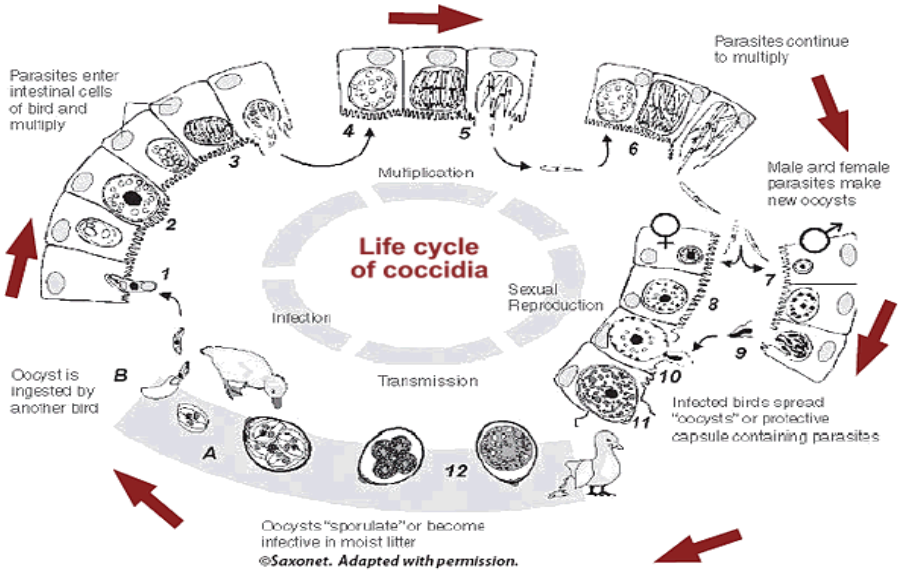


Figure 1: Life cycle of coccidia

2.6. Pathogenecity

Pathogenecity is related to the dose of infective oocysts received by the chicken and the strain of the parasite. The most common form of the disease is caecal coccidiosis, caused by *Eimeria tenella*. This normally occurs between 4 and 6 weeks. A small but sudden rise in mortality may occur and dead chickens will have an anaemic appearance. The outbreak tends to occur amongst a single group or house. It is very important to treat when the disease is first seen. Tissue damage and changes in the intestinal tract, as a consequence of infection, may allow colonization by other harmful bacteria, such as *Clostridium perfringens*, which causes necrotic enteritis (Immerseel *et al*., 2004). *Eimeria necatrix* attacks the small intestine, with the maximum involvement near the middle. The sporozoites penetrate the epithelium of the villi and migrate through the lamina propria towards the muscularia mucosa. Enroute most of them are engulfed by macrophages which transport them into the epithelium of the fundi of the intestinal gland. The invaded epithelial cell become hypertrophyed and migrate to the lumen of the gland fundus, meanwhile becoming first generation schizonts. The second generation schizonts are similar in form and behavior. On the 4th and 5th days aggregation of these schizonts appear as small whitish opacities. Later punctuate hemorrhage appear in the centre of the whitish areas. The unopened intestine thus presents a spotted appearance. The small whitish areas being intermingled with rounded, bright or dull red blotches of various sizes while transversely extending reddish streaks represent hemorrhages along the superficial vessels. There is profuse hemorrhage in to the lumen of intestine. *Eimeria necatrix* is unique among fowl coccidia in that, while the first two generation of schizonts develops in the small intestine, the merozoites generated by the second generation schizonts migrate to the caeca where they invade the epithelium and develop some into further generation of schizonts and some directly into oocysts. The disease may be acute resulting death after 5 to 7 days of infection and chronic where disease may linger for long time with a wasting illness. *Eimeria* oocysts are broadly ovoid, smooth and without micropyle. There are three asexual generation of merozoite. *E. necatrix* is also a common species. It first and 2 generation merozoite occur in the small intestine and its third generation merozoite and gamete are in the caecum. It is also highly pathogenic which causes the small intestine mucosa to become thick. This thickness remains after the coccidia are gone. The oocysts are oblong ovoid, smooth and without a micropyle. *E. acervutina* is perhaps the most common species. It occurs in the epithelial

cells of the villi and to a lesser extent, in the gland cells of the anterior small intestine. Some strains are only slightly pathogenic if a large number of oocysts are given. Its oocysts are ovoid, smooth and without a micropyle. There are four asexual generation of merozoite. *E. maxima* is also a common species. Its merozoitre occur in the epithelial cells of the villi of the small intestine and its gametes are displaced towards the centre of the villi and come to lie in their interior. Its oocysts are ovoid, smooth or somewhat roughened and without a micropyle. There are two asexual generation of merozoites. sporosoitos plays an important role in establishing infection, amylopectin is probably a source of energy to survive and to access, invade and develop in their host cell (Levine, 1983).

2.7. Clinical Signs

Infected chickens tend to huddle together, have ruffled feathers and show signs of depression. The chickens consume less feed and water, impaired feed conversion and droppings are watery to whitish or bloody. This results in dehydration and poor weight gain as well as high mortality. Mucoid to blood tinged exudates, petechial haemorrhages, necrosis, haemorrhagic enteritis and profuse mucosal bleeding in the caeca. The tissue damage in the intestinal tract may allow secondary colonization by various bacteria, such as *Clostridium perfringens* or *Salmonella typhimurium* (Helmbolt and Bryant, 1971; Arakawa *et al*., 1981; Baba *et al*., 1982). Infestation with *E. tenella* also increases the severity of *Histomonas meteagridis* infection in chickens (MeDougald, 2003).

Infected chickens will have ruffled feathers and a breakdown in intestinal function is the first recognizable symptom of coccidiosis and blood in the droppings may be observed. The extent of the pathogenicity depends on the species of parasite. The most virulent strains will cause diarrhoea and a sudden increase in flock mortality. Less virulent strains will result in poor growth and reduced feed efficiency. Hence the losses resulting from coccidiosis may be variable. There is normally bloody diarrhoea, anaemia, a reduction in feed and water intake (Williams, 2006). Death occurs in chicken mostly due to hemorrhage caused by large second generation schizonts stage of the *Eimeria* (Waxier, 1998). Depression, ruffled feather, reduction of feed and water intake takes place in the experimentally induced coccidiosis in chickens (Reid and Pitoais, 1965). Caecal or bloody coccidiosis is caused by *Eimeria tenella*. The parasites invades the caeca and adjacent of digestive tract, characteristic bleeding and cheesy cores noticed (Reid, 1972).

Coccidiosis causes reduction in egg production and lighter yolk colour. It also reduces plasma carotenoid level (Ruff *et al*., 1976). *E. tenella* causing weight loss, hemorrhagic enteritis and even death in young chickens (Levine, 1983). *Eimeria tenella* as the most pathogenic of all the chicken coccidia. It causes cecal hemorrhage after a moderate or severe infection and death occurs mostly on 5th or 6th day after infection (Tyzzer, 1990).

2.8. Pathology

2.8.1. Gross lesions

In moderate infections there is a thickening of the gut wall, a pinkish or blood tinged catarrhal exudates and in the mucosa, short, transverse red streaks, a millimeter or so in length, arranged in ladder like fashion in long rows down the lower intestine and in rectum may be found. In severe infections there is an extensive coagulation necrosis and sloughing throughout the entire intestinal mucosa, caseous cores may be found plugging the narrow portion of the caeca but the dilated portion of the caecal wall are only moderately affected (Levine, 1983). Small focal areas of denuded epithelium and focal area of necrosis in underlying connective tissue seen after second day. Enlargement and discoloration of the caeca with small areas of hemorrhages. On the 3rd day, further necrosis of denuded areas occured seperating such areas from the underlying connective tissue. Moreover spotted irregular focal hemorrhagic areas some larger in size appeared on the serosal surface. The lumen filled with blood and flakes of loosened ulcerated mucosa. Deeper layers contained large areas of congestion while the caecal wall was thickened. The connective tissue as well as the muscularis mucosa became necrotic and the underlying submucosa was edematous (Bertke, 1989). There are two types of diseases, hemorrhagic and catarrhal inflammations are found due to coccidiosis. In hemorrhagic type, lesions are distension of caeca with blood, blood clots and reddish brown contents. In catarrhal type, petechial spots seen through serosa associated with watery ingesta mixed with mucus. Due to *Eimeria necatrix*, the middle part of the small intestine is distended and crimson with petechiae seen through serosa. The intestinal contents are fluidy or curdy and mucoid mixed with streaky or spotted hemorrhage. Due to *E. acervulina*, less intense and moderate changes occur in small intestine. Grayish white pin point foci in the mucosa occur in the earlier part of the small intestine. Intestinal contents are liquid and mixed with mucous. Streaky hemorrhages are also observed. Mild catarrhal infection in the middle parts of small intestine due to *E.*

maxima. The intestinal wall found to be thickened and hyperemic with occasional pin point hemorrhage on the intestinal mucosa. Sharp lines of demarcation between affected and unaffected areas are noticed. The intestinal contents appeared to be curdy and tinted with blood streaks. The changes occur due to mixed infection are distention of entire length of small intestine with crimson appearance with hemorrhagic spots and grayish white foci seen through the serosa. The intestinal contents are reddish brown in color with blood clots and fibrins threads. Large masses of fibrin clots with blood streaks were are reported (Jagadeesh *et al.,* 1976). A change in the jejunal villus pattern to blunt shortened mucosal projections in chicks infected with *Eimeria acervulina*. The condition is probably due to an indirect effect of the parasites on the kinetics of the crypt epithelial cells (Poul, 2009). The lumen is filled with blood and pieces of loosened ulcerated mucosa. By 6th day, the lumen contents become hardened and speckled with a grayish core representing the clotted blood, mucosal debris. The gross lesions of *Eimeria necatrix* the serosal surface may be bright red and show numerous minute petachae. Inflammatory cell infiltrate the epthelium and produce an overall thickening of the intestinal wall followed by the pathogenic appearance of the whitish yellow plaques containing schizonts. Due to *Eimeria maxima* the zone in which the epithelial cells are parasitized is localized in the middle intestine which show hemorrhagic enteritis associated with thickening of the intestinal wall and some ballooning. The intestinal contents are brown, orange, pink or red brown with a very viscous mucous secretion present. The gross changes caused due to *E. maxima* are red pinpoint lesions may appear in the lower intestine, just above the junction of the caeca. By 4th day, intestine appears as whitish and hemorrhagic area increases in size appears in the lower small intestine and caeca. Caseous core may appear in the caeca and rectum. Swelling of intestine occurs and red pin point lesion turns to brown. The typical ladder like transverse lesions usually founded for *Eimeria acervulina*. In the duodenal and upper jejunal area represent light infection. Heavy infection causes coalescent in the lesions and thickening of the mucosa. Color of the intestine may be grayish yellow in light or moderate infection. Bright red congestion may occur in extremely heavy infection. The lesions in the lower small intestine, rectum and proximal area of the caeca are produced by *Eimeria brunetti*. In severe cases a coagulation necrosis produces a caseous erroted surface over the entire mucosa. The enlargement of caeca and small areas of hemorrhage. By 4th day, caeca is enlarged to three times of normal size, spotted irregular focal hemorrhagic areas appear on the serosal surface (Reid, 2002).

Eimeria tenella is the cause of so called caecal or bloody coccidiosis of chicks. Involvement of the caeea rather than of the small intestine is one of its characteristic features. The severity of this type of coccidiosis is attributable to the second generation schizonts which causes infected epithelial cells to increase tremendously in size and assume a migratory habit. Through pressure or otherwise there is produced sufficient degeneration of the blood vessels and surrounding tissues to result in bleeding into the caeca and the copious bloody discharge from the caeca. *Eimeria maxima* is far less lethal than *Eimeria tenella* and *Eimeria necatrix*. The lesions produced are dilation of the small intestine and thickening of the wall. The intestinal content is viscid mucus. Grayish, brownish or pinkish in color. Flecks of blood may be present. *Eimeria accervulina* is not a severe pathogen but commonest of all the poultry coccidia. Numerous gray, redish or whitish patches in upper half of the small intestine, reddish to brown contents in the intestinal lumen are seen. These patches are caused by forming oocysts (Becker, 1959).

2.8.2. Microscopic Lesions

The pathological changes caused by *Eimeria mivati* are petechial hemorrhage, infiltration of eosinophil, neutrophil, histiocyte and lymphocyte in areas near parasitized cells, The villi of the mucosa were destroyed and disorganised and there was no continuation in the lining epithelial cells of villi and proliferation of lymphoid tissue in the lower small intestine (Noyilla *et al*., 2007). The histopathological changes occurs due to *E. tenella* are distortion of normal architecture of intestine and desquamation of epithelium, enlargement of internal glands and developmental stages of parasite and cellular infiltration are also reported. The inflammatory cells are pseudoeosinophil, macrophages and lymphocytes. The histopathological changes due to *Eimeria necatrix* are the affection of superficial and middle third of intestinal mucosa and extensive hemorrhage. Inflammatory cells are macrophages, lymphocytes, pseudoeosinophils, mononuclear cells and cystic degeneration of the intestinal glands. The changes caused by mixed infection (*E. necatrix* and *E. accrvulina*) are extensive areas haemorrhage around the enlarged epithelial cells, infiltration of macrophages and lymphocytes, secretory vacuolation of glands, degeneration of epithelial cells, glands, intestinal villi and infiltration of inflammatory cell in the musculature and developmental stages of parasite is noticed almost in the entire thickness of mucosa (Jagadeesh *et al.,* 1976).

2.9. Immunity

Day old chicks do not normally acquire passive immunity from hens, although the potential of maternally transmitted antibodies as a means of control has been investigated (Smith *et al*., 1994a; Smith *et al.,* 1994b; Wallach *et al*., 1995). Chickens of all ages are susceptible. Although the risk of coccidial infection may increase with age and the effects of infection may be more serious in chicks (Rose, 1967; Rein, 1968). Chickens can develop immunity after infection, but this immunity is species specific, leaving birds susceptible to other *Eimeria* species. Immunity to *Eimeria* species is acquired gradually and is not complete until the birds are 7 weeks of age. It has been shown that immunity develops more rapidly to *E. maxima* than to some other species (Chapman and Saleh, 1999). Usually immunity will be acquired by a flock by "trickle" infection without the occurrence of clinical disease. However, if environmental conditions, such as wet litter, promote sporulation, birds that have not acquired immunity (typically 3-6 weeks) will succumb. Immunosuppressive diseases, such as Marek's disease, infectious bursal disease (IBD) and others, interfere with the development of immunity and infected chickens can be more susceptible to coccidiosis (Biggs *et al*., 1968).

2.10. Concurrent Infections Occurring During the Course of Coccidiosis

Coccidiosis is involved in primary or secondary disease in 35 percent cases in fowl (Poal, 1969). Early exposure to the Infectious Bursal Disease Virus increase the severity of caecal coccidiosis (Anderson *et al.,* 1977; Ahmed *et al.*, 1993; Singh *et al.,* 1994:

Chowdhury *et al*., 1996) and may decrease the effectiveness of some anticoccidial drugs (McDougald *et al*., 1979). Necrotic enteritis is exacerbated by the infection of intestinal species of coccidiosis (*E. acervulina, E. maxima and E. brunetti*) as shown in the experimental field studies involving the bacterium *Ctosrtidium perfengens* (Sen *et al.,* 1981). A close association between coccidiosis and Marek's Disease is often reported from the field observation. Experimental inoculation with ocysts of *E. mivati* and Marek's Disease did not increase the mortality to Marek's Disease (Brewer *et al*., 1969) but some decrease in immunity development to coccidiosis if Marek's Disease is introduced into some strain of chicken at the same time as the ccoidial ocysts (Biggs *et al*., 1968). During Coccidiosis, there can be other infection such as Reovirus infection. New Castle virus infection and infectious bronchitis virus infection (Biggs *et al*., 1968).

2.11. Economic Importance

Losses due to chicken coccidiosis had been estimated 1-40 million dollars in the United States. Because of the importance of these protozoan parasites a great deal of research or therapeutics, pathogenocitiy, host parasite relationship and species differentiation were conducted (Zimniermann, 1957). Coccidiosis is an old parasitic disease, prevalent all over the country and has a significant impact on poultry production. The economic loss to poultry industry has been estimated considering the major economic parameters. The estimation has revealed that commercial poultry industry is a major sufferer due to coccidiosis where in 95.61 percent of the total economic loss occurs due to the disease. A comparison across economic traits has revealed that loss is maximum due to reduced body weight gain, followed by increased FCR (23.74%) and chemoprophylaxis (2.83%) in the total loss due to coccidiosis in poultry industry of India. The overall comparison of economic traits for all the types of poultry sector it has shown that reduced body weight gain and increased FCR are the major parameters from which 68.08 per cent and 22.70 percent annual loss has occurred in the total loss from coccidiosis in India during the year 2003-04. The total loss due to coccidiosis has been found to be of Rs 1.14 billion (approx) for the year 2003-04. The study has observed that generation of this data across different geographical regions will be helpful to conclude about the global economic loss due to coccidiosis in the poultry industry (Bera *et al*., 2010).

CHAPTER III

MATERIALS AND METHODS

3.1. Experimental Chickens and Research Area

The chickens of different commercial poultry farms were considered as experimental chickens. Coccidia outbreaks in the small scale commercial poultry farm were investigated at Bogura district of Bangladesh and the laboratory examinations were conducted at the Department of Pathology and Parasitology under Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

A total of 95 farms were visited; 343 diseased and dead chickens were examined out of which 52 chickens were found to be positive for coccidiosis. The number of chickens in the farms was variable ranging from 250 to 1250 and they were reared on litter. A detail farm history in relation to the incidence of disease including housing system, location of poultry farms, sources of chickens, age and population of the chickens per flock, rearing system, litter material, feeding and watering system, biosecurity of the farms, previous history on coccidia outbreaks, intervals between the batches, rearing of one more batches in the same farm at the same time, etc. were also recorded. The chickens affected with Coccidiosis were submitted to the Pathology laboratory for the diagnosis and treatment were the principal experimental chickens and some affected chickens were also collected physically.

3.2. Research Period

The duration of experiment was 6 months from July to December, 2018.

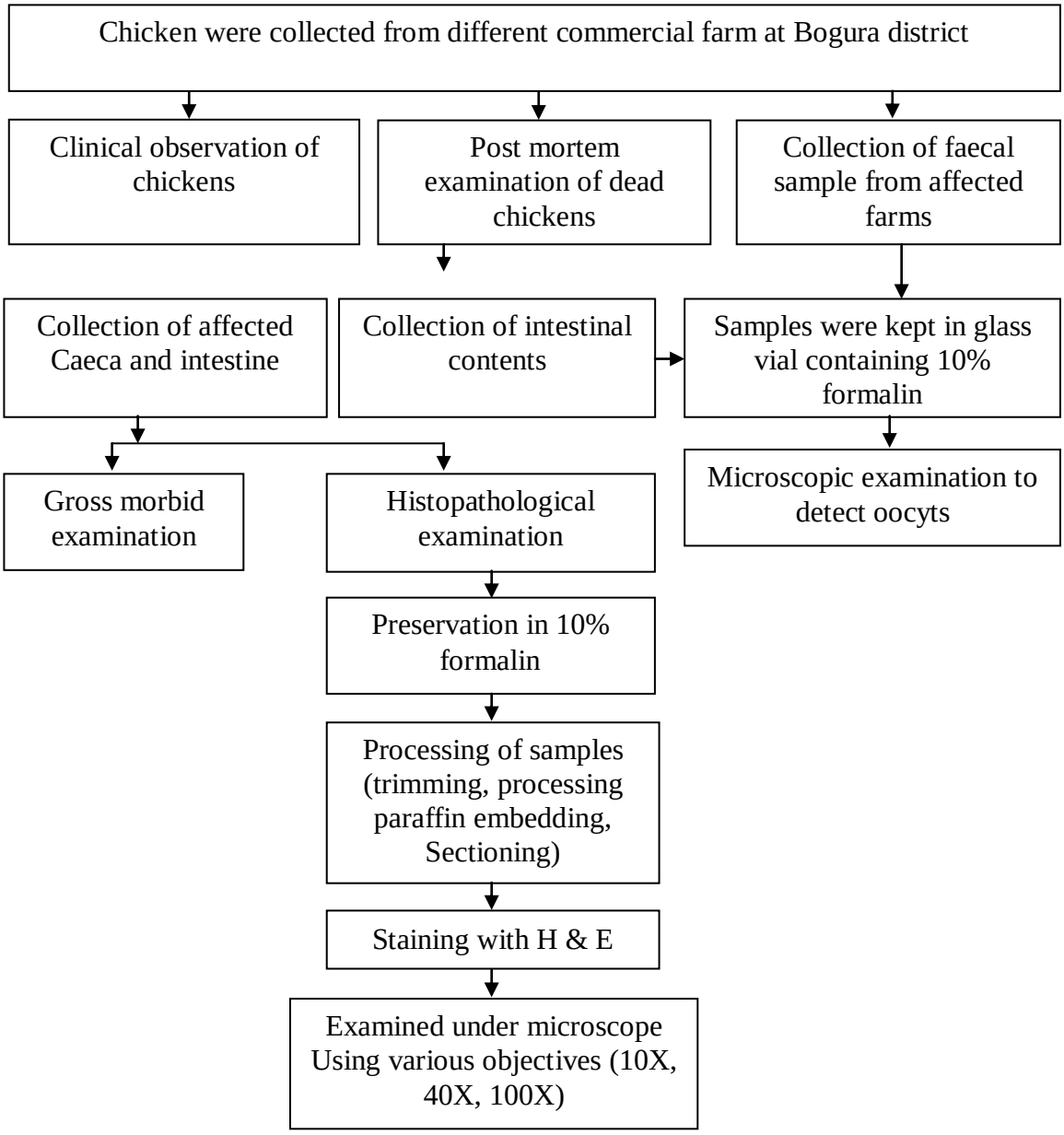
3.3. Sampling Occasion

There was no scheduled sampling occasion. Chickens affected with Coccidiosis were collected and examined when submitted to the laboratory only as well as the collection during the physical visit.

3.4. Parameters of the Present Study

- Clinical Examination of affected chickens.
- Fecal examination for oocysts determination.
- Necropsy to detect lesions of coccidiosis in suspected chickens.
- Histopathological examination of caecum and other intestinal parts.

3.5. Experimental Layout



3.6. Cleaning and Sterilization of Required Glassware

Reagent bottles, glass bottle, spirit lamp, test tubes, glass tubes, glass slides, cover slips, beakers, pipettes,measuring cylinders etc. were used in this study. The conical flask, measuring cylinder, beakers, glass slides, cover slip, for slide preparation for histopathological study and staining of organisms after smear and pipettes, reagent bottle, glass tubes for different biochemical tests. New and previously used glassware were collected and dipped in 2% sodium hypochlorite solution and left there until cleaned. After overnight soaking in a household dish washing detergent solution, the glassware were cleaned by brushing and washed thoroughly in running tap water and

rinsed three times in distilled water. The cleaned glass wares were then dried on a bench at room temperature or in an oven at 50-70°C.

3.7. Clinical Examination of Affected Chickens

The general health condition and age of the chicken were recorded. The clinical signs were recorded during the physical visit of the affected farms and the farmer's complaints about the affected chickens were also considered.

3.8. Necropsy Findings of Suspected Chickens

The necropsy was done on the suspected dead and diseased chcikens taken from different upazilla of Bogura district. At necropsy, gross morbid changes were observed and recorded carefully by systemic dissection. The collected samples were preserved at 10% formalin for the histopathological study.

Equipment and appliances for necropsy

- Sample (chickens)
- Scissors (3)
- Forceps (4)
- Bone cutting saw
- Scalpel
- Chisel
- Gloves
- Musk
- 10% formalin

Procedures

- At first the chicken was wet in a detergent solution thoroughly to lessen the chances of feathers floating around the area while the examination.
- The chicken was laid on a pad of newspaper on post mortem table. The paper served to absorb most blood and fluid, and provided a convenient wrapper for the carcass after examination.
- The chicken was positioned in such way so that the legs and feet were facing the examiner.

- Then an incision was given on skin in between the thighs towards the back and through skinning was done to observe paleness condition of carcass fot detection of anaemia.
- Body cavity of chicken was opened and the liver, spleen, gizzard, proventriculus, and other unnecessary organs were detached to facilitate the examination of intestinal parts.
- Segments of the intestines, caecum, and colon were observed carefully for important post mortem lesions.
- Then the parts opened longitudinally by knife or scissors to observe the colour, consistency and appearance of intestinal contents and mucosal surfaces gradually.
- The caecal junction and the caecum at either side were opened and were examined in similar manner.

Gross lesions

Gross morbid lesions of different organs were registered during the course of necropsy of the chickens.

3.9. Histopathological Examination

During necropsy, various organs having gross lesions were collected, preserved at 10% formalin, processed for the histopathological study. Formalin fixed samples of the small intestine, large intestine and caeca from the diseased and dead chicken were processed for paraffin embedding, sectioning and staining with haematoxylin and eosin according to standard method (Luna, 1968) for histopathological study.

Details of tissue processing, sectioning and staining were given below.

3.9.1. Equipment and appliances

Sample (duodenum, jejunum, colon, caecum etc)

- Chloroform
- Alcohol
- Paraffin
- 10% formalin
- Tape water

- Xylene
- Hematoxylin and Eosin Stain
- Distilled water
- Clean Slides
- Cover slips
- Mounting media (dvx)
- Microscope

3.9.2. Processing of tissues and sectioning

- The tissues were properly trimmed to obtain a good cross section of the tissue.
- The tissues were washed under running tap water for overnight to remove the fixative.
- The tissues were dehydrated in ascending grades of alcohol using 50%, 70%, 80%, 90% alcohol, and three changes in absolute alcohol, for 1hr in each.
- The tissues were cleared in two changes in chloroform, 1.51w in each.
- The tissues were embedded in molten paraffin wax at 56°C for two changes, 1.51w in each.
- Paraffin blocks containing tissue pieces were made using templates and molten paraffin.
- The tissues were sectioned with a microtome at 5mm thickness, which were allowed to spread on warm water bath (42°C) containing small amount of gelatin and taken on oil and grease free glass slides. The slides were air dried and kept in cool place until staining.

3.9.3. Hematoxylin and Eosin Staining Procedure

Preparation of Harris' hematoxylin solution

Hematoxylin crystals	5.Og
Alcohol (100%)	50.0 ml
Ammonium or potassium alum	100.0 g
Distilled water	1000.0 ml
Mercuric oxide (red)	2.5 g

Hemoatoxylin was dissolved in alcohol and alum in water by heat. The two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat. Mercuric oxide was added to the solution slowly. The solution was reheated to a simmer until it became dark purple, and then the vessel was removed from heat and immediately plunged into a basin of cold water until it became cool. 2-4ml glacial acetic acid was added per 100 ml of solution to increase the precision of the nuclear stain. Before use, the prepared solution was filtered.

Preparation of eosin solution

1% stock alcoholic eosin

Eosin Y, water soluble	1g
Distilled water	20 ml
95% alcohol	80 ml

Eosin was dissolved in water and then 80 ml of 95% alcohol was added.

Working eosin solution

Eosin stock solution	lpart
Alcohol, 80%	3 parts

0.5m1 of glacial acetic acid was added to 100 ml of working eosin solution just before use.

Staining protocol

- Deparaffinization of the sectioned tissues was done in xylene (three changes; three minutes in each).
- Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 minutes in each change; 95% alcohol for 2 minutes; 80% alcohol for 2 minutes; 70% alcohol for 2 minutes) and distilled water for 5 minutes).
- The tissues were stained with Harris' hematoxylin for 10 minutes.
- The sections were washed in running tap water for 10 minutes.

- Then the staining was differentiated in acid alcohol (1 part HCI and 99 parts70% alcohol) 2-4 dips.
- The tissue sections were then washed in tap water for 5 minutes and dipped in ammonia water (2-4 times) until sections became bright blue.
- The sections were stained with eosin for 1 minute and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each change; absolute alcohol 3 changes, 2-3 minutes in each).
- The stained sections were then cleaned by 3 changes in xylene, 5 minutes in each change and finally the sections were mounted with cover slip using DPX.
- Then the images of the stained section were taken by digital camera (Canon 1XY, 16.1 Mega pixels, Japan).

3.10. Parasitological Examination of Faeces

3.10.1. Collection of faeces

Faecal samples were collected directly from anus with spatula or freshly fallen faeces from the affected flocks.Faecal sample was collected during the postmortem examination of the chickens.

3.10.2. Microscopic examination of faeces

The faeces were examined in two methods

a) Direct Smear technique
b) Floatation technique

a) Direct Smear technique

Procedures

- Approximately 3g of faeces was taken into a container.
- Small amount of faeces was taken on a glass slide and a drop of water.
- Then the faeces was spread thinly with a rod stirrer.
- Then the cover slip was placed on slides.
- The slides were examined under microscope for detection of oocysts in low (10x) and high magnification (100x).

b) Floatation technique

Procedures

- The faecal samples were examined by floatation technique under standard protocol (Fowler and Miller, 1999).
- Approximately 3g of faeces was taken into a container.
- The floatation fluid was added into the container which containing faeces
- The faeces were mixed thoroughly with the flotation fluid with stirring device.
- Then the faecal suspension was poured through a tea strainer into another container.
- The container was leaved to stand for 10 minutes
- The test tube was filled with faecal suspension up to full
- Then the test tube was stand in a test tube rack to stand for some minutes
- A cover slip was placed on top of the test tube
- Then the cover slip was placed on the slides
- The slides were examined under microscope for detection of oocysts in low(10x) and high magnification (40x,60x, and 100x)

3.11. Photography

All images related to the present study were taken directly from microscope using different objectives manipulation of zooming system of a digital camera (Canon, 1XY,

16.1 Mega pixels, Japan). The images were provided following minute modification for the better illustration of the study.

3.12. Methodology

All data was recorded and stored in Microsoft Excel a Spreadsheet and collected data was analyzed with Statistical Package for Social Science (SPSS) software (version-22.00).

CHAPTER IV

RESULTS

Pathological investigation of chicken coccididosis in small scale commercial farms at Bogura district was studied and different clinical, necropsy and histopathological conditions were recorded during the study period.

4.1. Clinical Findings of Affected Chickens

The present clinical examination different type of clinical signs caused by different species of *Eimeria.* During clinical examination following clinical signs were depression and ruffled feather, blood in faeces, anaemic carcass, and attachment of faeces around vent. Bloody diarrhoea is considered to be a most important clinical signs in the examined chicken. Prevalence of coccidiosis at different commercial farms are shown in table 2. Age related prevalence of coccidiosis in chicken is shown in table 3. Variety related prevalence of coccidiosis in chicken is shown in table 4.

Table 2: Prevalence of coccidiosis at different commercial farms

Location of the farm (Upazila)	No. of sample	No. of positive sample	Percentage (%)
Sadar	45.00	6.00	13.33
Gabtali	35.00	3.00	8.5
Sariakandi	30.00	4.00	13.33
Sherpur	41.00	6.00	14.63
Shajahanpur	25.00	5.00	20.00
Shibganj	42.00	7.00	16.67
Adamdigi	30.00	6.00	20.00
Dhunat	37.00	6.00	16.21
Sonatala	32.00	5.00	15.62
Dhupchachia	26.00	4.00	15.38
P value	0.98 (NS)		

NS Means non-significant (P>0.05)

Table 3: Age related prevalence of coccidiosis in chicken

Age group	No. of sample	No. of positive sample	Percentage (%)
0-4 weeks	135	26	19.25
5-6 weeks	70	15	21.42
7-8 weeks	85	7	8.23
>8 weeks	53	4	7.5
P value	0.024*		

* Means significant at 5% level (P<0.05)

Table 4: Variety related prevalence of coccidiosis in chicken

Type	No. of sample	No. positive sample	Percentage (%)
Broiler	160	33	20.6
Sonali	105	11	10.47
Layer	78	8	10.25
P value	0.031*		

* Means significant at 5% level (P<0.05)

Figure 2: Attachment of feces around vent

Figure 3: Feces mixed with blood

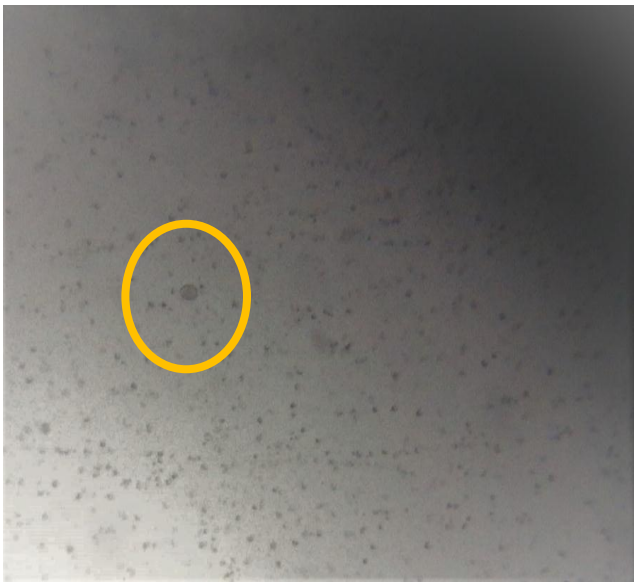


Figure 4: Oocyst detection, Oocyst of *Eimeria* sp. (Yellow ring)

4.2. Necropsy Examination

Necropsy findings in different intestinal regions of chicken were detected such as enlargement and discoloration of caecum with numerous haemorrhage spots, blood mixed intestinal contents in the intestinal lumen, reddish to brown contents in the intestinal lumen, pin point haemorrhage on the intestinal mucosa, profuse haemorrhage on intestinal wall and massive haemorrhage on intestinal mucosa.

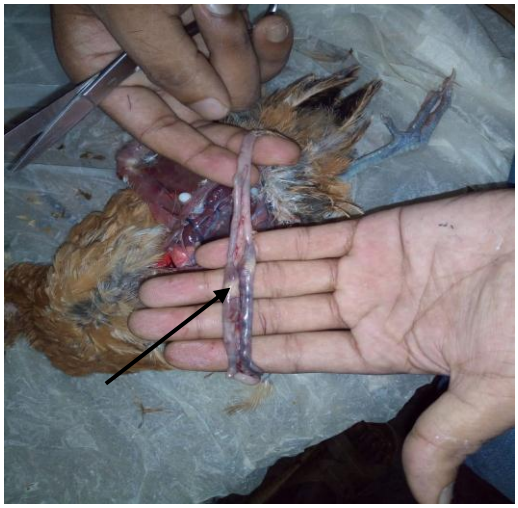


Figure 5: Deeper layers contained large areas of congestion

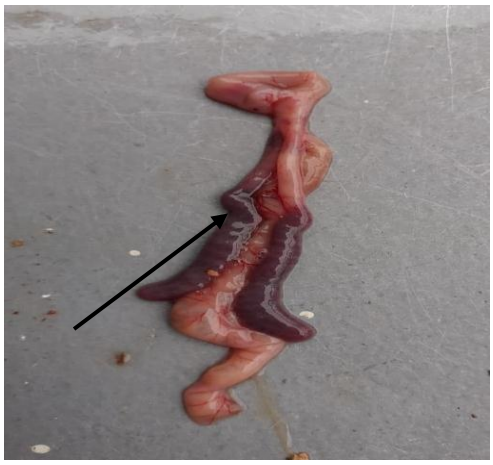


Figure 6: Pinkish or blood tinged catarrhal exudates

4.3. Histopathological Study

In this study, destruction of normal architecture of caecum (Fig. 7) and proliferation of fibrous connective tissue of liver (Fig.8). The villi of the mucosa were destroyed and disorganized and there was no continuation in the lining epithelial cells of villi.

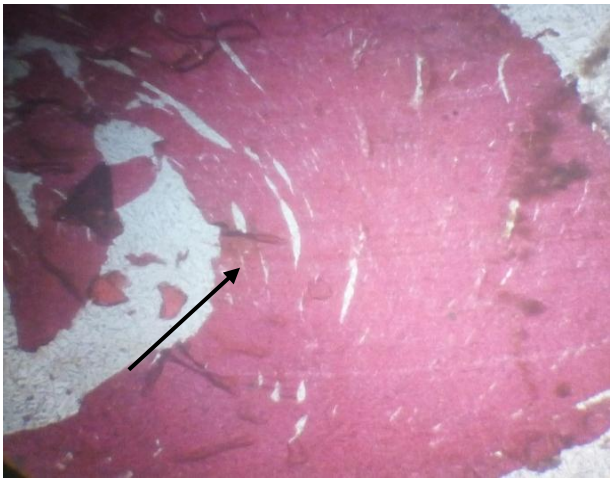


Figure 7: Destruction of normal architecture of caecum

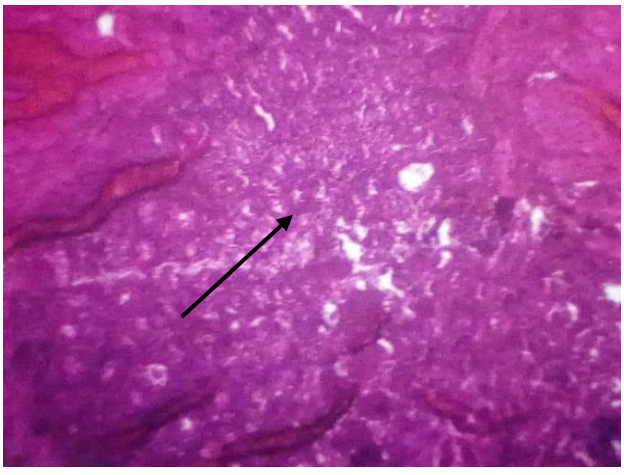


Figure 8: Proliferation of fibrous connective tissue of liver

CHAPTER V

DISCUSSION

This study was undertaken to investigate the pathological condition of Chicken Coccidiosis from the outbreak at small scale commercial farm in different upazila of Bogura district.

5.1. Overall Prevalence of Coccidiosis in Chicken

The study was conducted in different small scale commercial farm in different upazila in Bogura district. Total 95 farms were visited. Different species of *Eimeria* were found to be prevailed in those farms. Total 338 diseased and dead birds were examined out of which 52 chickens were found to be positive for coccidiosis. There was a significant relationship found among the prevalence of coccidiosis in different upazila of Bogura District. The prevalence was maximum in shajahanpur upazila (20.00%) and also Adamdigi which is statistically similar to both Sadar (10.33%) and Sariakandi (10.33%). On the other hand, the minimum prevalence of coccidiosis was 8.5% which is founded in Gabtali upazilla .This observation is similar to those reported in other authors where the incidence of coccidiosis was recorded 9.40% by Bhattachrjee *et al*., 1996; 9.46% by Islam *et al*., 2003 and 8.71% by Salam *et al*., 2011 respectively. In West Bengal 85 (10.91%) cases of coccidian is recorded by Bhattacharya Pramanik. 1987. These variations among the present and earlier studies might be due to rearing practice, Geographical location, study period, differences in sample collection techniques, and Deviation in identification procedure

5.2. Age related prevalence of Coccidiosis in chicken

Young chickens are more susceptible and more readily display signs of disease, whereas older chickens are relatively resistant as a result of prior infection. Typically, the disease is seen in chickens of 3-6 weeks old before they have acquired immunity. The proportional mortality rate of coccidiosis in different age group were 19.25%, 21.42%. 8.23% and 7.5% in 0-4weeks, 5-6weeks. 7-8weeks and above 8week respectively which is similar to the observation by Kamath. 1987; Rose, 1999; Humphrey, 1973 and Kogut *et al*., 2005. The exact cause of higher prevalence of protozoa in young than adult chicken cannot be explained but it can be hypothesized that younger chickens have less developed immune system compared to adults.

5.3. Variety related prevalence of coccidiosis in chicken

The present results showed that variety of chicken had an influence on the prevalence of coccidiosis. Under the present study the higher prevalence of coccidiosis broiler (20.6%) than sonali (10.47%) and layer (10.25%) which was not statistically significant. The present findings contrast with the observation of Etuk *et al*., (2004) who recorded a higher prevalence of coccidiosis in adult layer chicken in other age categories which is different from this study may be due to location, season, age difference, sex, breed and other managemental factors.

5.4. Clinical Examination

Clinical manifestation of chickens naturally infected with Coccidia was studied. During this investigation the common clinical manifestations in the chicks suffering from natural Coccidiosis were found as Bloody diarrhoea, attachment of faeces around vent (Fig 2), and blood mixed with feces (Fig 3).These findings are also similar by Reid and Pitoais, 1965 and Williams, 2006.

Weight loss, reduction in egg production, damp litter and death occurs mostly on 5th or 6th day after infection were also found in this observation. Similar findings were reported by Tyzzer, 1990; Waxier, 1998; Ruff *et al*., 1976 and Levine, 1983.

5.5. Necropsy Examination

A total number of 23 dead and sick chickens suspected to be infected with coccidiosis were collected from small scale commercial farm in Bogura district and subjected to postmortem examination. Gross pathological changes of the various organs of the affected chickens were studied. At necropsy, the major pathological lesions were deeper layers contained large areas of congestion (Fig 5), pinkish or blood tinged catarrhal exudates (Fig 6). These gross lesions are also reported by Bertke, 1989; Becker, 1959 and Reid, 2002.

Thickening of intestinal wall than normal. Hemorrhage and extravasations of blood within the intestinal lumen. profuse congestion and pin point hemorrhage on intestinal mucosa, hemorrhagic enteritis and blood-tinged exudates. These observation is similar to those reported by Poul, 2009; Jagadeesh *et al*., 1976; Arakawa *et al*., 1981 and Levine, 1983.

5.6. Histopathological Study

The histopathological change founded in the present study were destruction of normal architecture of caecum (Fig 7), Proliferation of fibrous connective tissue of liver (Fig 8). The villi of the mucosa were destroyed and disorganised and there was no continuation in the lining epithelial cells of villi. This observation is similar to those reported by Noyilla *et al*., 2007 and Jagadeesh *et al*., 1976.

CHAPTER VI

SUMMARY AND CONCLUSION

The present study was conducted to clinic-pathological investigation of chicken coccidiosis based on clinical, parasitological, necropsy, and histopathological feature. The study was conducted in different upazila in Bogura district. Total 95 farms were visited among which 343 diseased/dead chickens were examined out of which 52 chickens were found to be positive for coccidiosis. Different type of *Eimeria* were found to be prevailed in those poultry farm. The proportion incidence of coccidiosis is lower because the farmers are intensively aware of coccidiosis and other parasitic disease now a day. They usually use coccidiostats routinely. The proportional mortality rate of coccidiosis in different age group were 19.25%, 21.42%. 8.23% and 7.5% in 0-4weeks, 5-6weeks. 7-8weeks and above 8week respectively.

The clinical signs of the affected birds were more or less similar to signs generally developed due to the infection with coccidiosis and clinically characterized as bloody diarrhea, anaemia, depression, ruffled feather, reduction of feed and water intake, drooping wings. At necropsy, deeper layers contained large areas of congestion, pinkish or blood tinged catarrhal exudates. Histopathologically, destruction of normal architecture of caecum, proliferation of fibrous connective tissue of liver. The villi of the mucosa were destroyed and disorganised and there was no continuation in the lining epithelial cells of villi.

From the above facts and findings, it could be concluded that-

- Outbreaks of coccidiosis in the commercial poultry farm is lower
- The farmers are intensively aware of coccidiosis now a day and they usually use coccidiostats routinely.

On the basis of this study it is assumed that although coccidiosis is a serious problem at poultry industry in Bangladesh, it possible to control under routne preventive and control measure which is prime essential for substantial improvement in poultry production.

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