PREVALENCE AND PATHOLOGY OF AVIAN COCCIDIOSIS AT DIFFERENT UPAZILA IN DINAJPUR DISTRICT

A Thesis

By

SHAKILA REZA
Registration No. 1305076
Semester: January - June, 2014
Session: 2013-2014

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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ABSTRACT

The study was intended to investigate the prevalence and pathological status of avian coccidiosis at different upazila like Sadar, Chirirbander, Parbatipur, Fulbari, Birampur, Nawabgonj in Dinajpur district from January to June, 2014. A thorough clinical and necropsy examination was done and 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 354 diseased and dead birds of 12 farms were examined. In which 31 (9.65% in broiler and 7.10% in layer) birds found to be positive for coccidiosis. The propotional mortality rate of coccidiosis were 10.66%, 9.33%, 6.17% 2.1%, respectively in age group of 0-4 weeks, 5 - 6 weeks, 7 - 8 weeks and above 8 weeks. The mortality rate was hight in 0-4 weeks age group (10.66%) and lowest in above 8weeks age group (2.1%). The clinical signs of the affected birds were depression, ruffled feather, bloody diarrhea, anaemia, drooping wings, paler comb and wattle. At necropsy, enlargement and ballowing shape of caecum with pin point hemorrhage on intestinal mucosa and fresh or clotted blood were found in the intestinal lumen. Histpathologically**,** the mucous membrane was found to be severely damaged and there was no continuity of mucosal layer of intestine, distortion of architecture and desquamation of lining cells were present. Infiltration of inflammatory cell in the musculature was also observed. The villi of the mucosa were destroyed. Disorganization of the lining epithelium was also found. The bio-safety measures, farmers knowledge and protection programs against the disease did not comply with the approved standards. Thus bio-safety measures, vaccination and proper treatment must be done to improve the management of coccidiosis in poultry farms of Dinajpur district.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
&	and
µm	micrometer
AC	Avain Coccidiosis
CV	Co-variance
ELISA	Enzyme Linked Immunosorbent Assay
et al.	And his associates
etc.	Etectera
FAO	Food and Agricultural Organization
Fig.	Figure
GIT	Gastrointestinal Tract
Gm	gram
H & E	Hematoxylin and Eosin
HSTU	Hajee Mohammad Danesh Science and Technology University
lbs	Pounds
LSD	Least standard deviation
Mg	Miligram
min	Minute
ml	Mililiter
MS	Master of Science
N	Normal
nm	Nanometer
No.	Number
^{o}C	Degree celsius
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PM	Postmortem
PPS	Department of Pathology and Parasitology
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
WHO	World Health Organization

CHAPTER I

INTRODUCTION

Poultry are kept in backyards or commercial production systems in most areas of the world. Compared to a number of other livestock species, most of the people are related with the production, marketing, and consumption of poultry products. For these reasons, poultry products have become one of the most important protein sources for people throughout the world. The total number of poultry in the world has been estimated by the Food and Agriculture Organization of the United Nations (Anders and Jorgen, 1998) as of 14,718 million, with 1,125 million distributed throughout the Africa, 1,520 million in South America and 6,752 million in Asia, 93 million in Oceania, 3,384 million in North America and 1,844 million in Europe. Bangladesh is one of the most densely populated country in the world with 152.5 million people (PDEU, 2011). A total of 5 million people are engaged in poultry sector (Saleque, 2006) with about 1,10,800 small and large scale poultry farms in this country (Anon, 2006). The increasing demand and economic aspect has created a lot of interest among the people to raise poultry either through backyard or intensive commercial farming system.

Poultry farming in Bangladesh has grown as an emerging and prospective industry and many landless farmers are found to involve with poultry rearing (Huque, 2001). At present chicken contributes 51% of total meat production in Bangladesh and per capita annual consumption of meat is 5.99 kg against the universal standard 80 kg per head (Raha, 2007). Traditional backyard poultry keeping with flock size of 5-20 birds, with almost zero financial input is quite popular amongst rural population comprising of farm women, landless labours and marginal farmers. It contributes to nearly 30% of national egg production (Singh *et al*. 2009). There is a vast need for developing poultry farming both in rural and urban areas for the fulfillment of protein supply. The average quantity of protein uptake by people is insufficient per head per day where as desirable requirement is decreasing daily per head day by day. Amongst food animals, poultry ranks high in their ability to convert feed into high

energy food products (meat and eggs) for human consumption. However poultry production is an easy and efficient way of producing animal protein, with less capital investment relatively more profit could be earned. 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 2006).

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). Among the different diseases, parasitic infection brings a great threat to poultry industry like Coccidiosis which is a common and fatal disease in poultry. Commercial poultry production has increased manifold during last decade but at the same time, coccidiosis which was primarily a sporadic disease in 1976 and has become a high occurrence of disease in 1986 (FAO/WHO/OIE, 1976, 1986). The coccidia of the genus *Eimeria* is an obligatory intracellular parasite with a complex life cycle. The availability of a suitable host is probably the only limitation to the distribution of coccidia. *Eimeria* is distributed throughout the world (Macpherson, 1978).

Intestinal coccidiosis, caused by various species of *Eimeria*, is an economically important disease of poultry (Zhang and Zeng, 2005). *Eimeria spp*. are belonging to the phylum Apicomplexa causing coccidiosis of farm animals and birds. *Eimeria tenella* is the most important species, as it causes caecal coccidiosis in chickens (Shirley, 1986). *Eimeria tenella* primarily invades and resides in the linings of caeca of exposed chickens (Vervelde and Vermeulen, 1995 and Yun *et al.*, 2000).

Temperature and moisture are important factors in the epizootiology of coccidiosis and faulty waterers have been identified as one source of excess moisture (Davies and Joyner, 1955). The optimum temperature for rapid sporulation of oocyst of different species of *Eimeria* has been reported to be from 28 to 30^0 celcius (Edgar, 1955). The hot and humid environment of poultry houses in Bangladesh provides an ideal condition for the sporulation of the oocyst of coccidia. Together with the high reproductive potential of the *Eimerian* parasites, they can help to build up of large

number oocysts in litter in a relatively short period of time. The usual practice of changing litter after each avian crop apperently removes most of the oocysts, but is not effective in domination of the parasites (Long, 1973).). A preliminary report on the occurrence of *Eimeria tenella*, *Elmeria necatrix* and *Eimeria maxima* as determined by the fecal examination of chicks from Bangladesh Agricultural University Poultry Farm was made by Mondal and Qadir, (1978). The occurrence of *Eimeria acervulina* and Eimeria brunetti in poultry in Bangladesh was reported for the first time by Karim and Trees,(1990). Srinevasan ,(1959) reported 90 to 100 percent mortality in chicken to be associated with coccidiosis in India. The mortality in young birds is predominant features. In adult also poor rate of weight gain or loss of egg production was observed (Levine, 1961).

Coccidiosis has also become a subject of growing interest as it causes significant economic loss in the poultry industry throughout the world. Considerable studies are being conducted to determine its economic importance associated with epizootiological factors and method of control of the disease. Unfortunately no figure is available on the economic losses due to coccidiosis in poultry in Bangladesh. Coccidia appears to be ubiquitous in distribution. The true picture of the prevalence and pathology of coccidiosis in avain species has not been found properly in this country. So the present study on prevalence and pathology of coccidiosis in poultry was undertaken with the following aims and objectives in view:-

- To determine the prevalence of coccidiosis in broiler and layer
- To study the clinical feature, necropsy and histopathology in GIT of infected birds
- To determine the mortality percentage in relation to age of birds due to avain coccidiosis

CHAPTER II

REVIEW OF LITERATURE

Available literature for the pathological determination of avian coccidiosis in chicken was reviewed with a brief overview on the history, epidemiology, oetiology, pathogenesis, pathology, clinical manifestations, life cycle, economic importance, treatment and control against avian 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*. Coccidiosis causes mortality, malabsorption, inefficient feed utilization, impaired growth rate in broilers and reduced egg production in layers (McDougald, 2003; Lillehoj *et al.*, 2004). It affects many species of mammals and birds, and is of great economic significance in farm animals, especially poultry.These diseased condition most commonly occurs under intensive rearing, where pathogenic populations of the causative agent may build up Avian 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 (Shirley *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 bile (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*, *Eimcria necatrix, Eimeria acervulin*a, *Eineria brunetti and Eimeria mitis.* Chicken are susceptible to at least 11species of coccidia **(**Information Fact Sheets 2009). *E. tenella* and *E. necatrix* are the most pathogenic species. (*Soulsby E.,* 1982**;** Lillehoj H. and Trout J, 1993).

2.3.2 Morphology

Eimeria spp. are frequently described from the morphology of the oocyst, a thick-walled zygote shed in faecal material by the infected host. Oocysts are enclosed in a thick outer shell and consist of a single cell that begins the process of sporulation to yield the infective stage in about 48 hours. Infective oocyst contains four sporocysts,

which in turn contain two sporozoites (Fig 1) (McDougald, 2003)

A membrane consists from three layers (one layer of lipoprotein between two layers of protein) locomotion by contraction. *Eimeria* spp. secretes enzymes to destroy host cell membrane and gets oxygen results from digest nutrients. Average of oocyst dimensions is 23 x 19 micrometer (µm). (Altaif K., 1986)

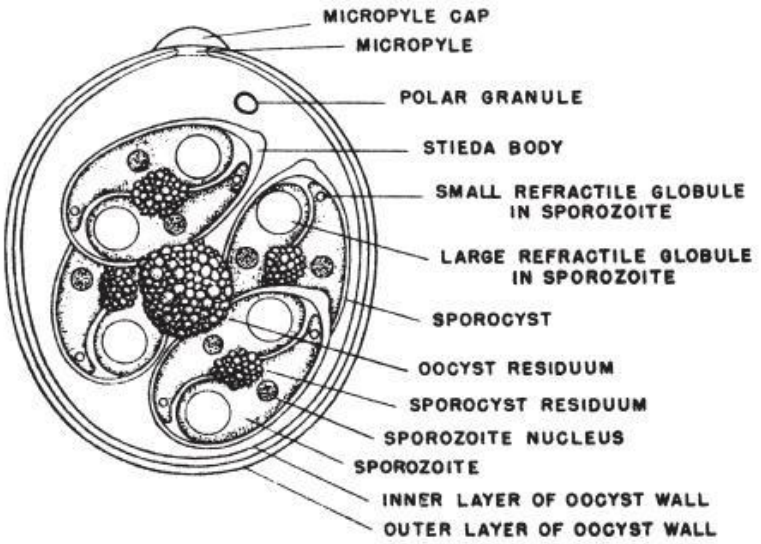

Fig. 1 Diagram of sporulated oocyst of genus *Eimeria*

2.3.3. 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, whilst *E. praecox* is regarded as the least pathogenic (McDougald, 2003; Shirley *et al*., 2005; Conway and McKenzie, 2007; Taylor *et al*., 2007). Most common spesies in Bangladesh *Eimeria tenella*, *Eimeria maxima*, *Eimeria necatrix*, *Eimeria acervulin*a and *Eineria 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 commonly 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, 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 behavioural characteristics. The large number of oocytes produced by infected birds 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 feces, the presence of these can have little or no relationship to an impending or existing infection (Joyner, 1978).

2.3.4 Most pathogenic species

E. tenella and *E. necatrix* are the most pathogenic species. (Soulsby E., 1982**;** Lillehoj H. and Trout J., 1993) in chickens. Infection with *E. tenella* can be recognised 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 **(**Levine, 1983; Tyzzer, 1929). 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 unthriftiness hemorrhagic exudate. 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 mucosol damage when compared *Eimeria mivati*, *Eimeria necatrix* and *Eimeria maxima* (Witlock *et al.*, 1977)

2.4. EPIDEMIOLOGY

2.4.1. Geographical distribution and prevalence of Coccidiosis

Coccidiosis is worldwide distributed (Macpherson, 1978). The prevalence of coccidiosis is worldwide and can be found in almost every commercial poultry flock (McDougald and Reid, 1977; Cox, 1998). 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 farmer are intensely aware of coccidiosis and other parasitic disease now a days. In Pakistan whereas in layers and breeders, *E. tenella* showed the highest prevalence, 38.88% and 65% respectively **(**Khan and Nasir, 2006) In the same geographical areas, *E. tenella* was the most prevalent species (Awais *et al.*, 2012), except in Iran where as in Europe, Australia and North America the most prevalent species was *E. acervulina* (Shirzad *et al.*, 2011). In Africa, Middle East and Asia the most frequent species reported in birds are *E. brunette* (between 10 and 60%) and *E. necatrix* (4–30%) (Lee *et al.*, 2010). The prevalence in Bangladesh were recorded 9.40% (Bhattachrjee *et al.*, 1996) and 39.2% of the birds (out of 337) were affected with coccidiosis (Islam *et al.*, 1996) respectively. The prevalence of coccidiosis in chicken in Bangladesh were 9.17%

(Giasuddin *et al.*, (2003). In West Bengal 85 (10.91%) cases of coccidlosia 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 (Jian Jun Zhang *et al.*, 2012). 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 *Eimeria anseris*. A large number of specific coccidia have been also reported. The most pathogenic coccidial infection of ducks is *Tyzzeria perniciosa*, which causes haemorrhagic 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 react preinfection production status (Williams, 1996).

2.4.4 Susceptible Age

Young birds 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 birds 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, 1967). Day 5 as the most severe stage of infection to histological and ultrastructural changes and decrease in nutrient absorption (Humphrey, 1973). Two week old chickens are susceptable to *E. acervulina* (duodenum), *E. maxima* (jejunum), *E. brunetti* (ileum) and *E. tenella* (caecum) resulting weight loss, intestinal lesion scores (Kogut and Powell, 1993). The higher rate of coccidiosis is determined in >6 weeks' age groups and all ages of poultry are susceptible to infection but usually resolves itself around 6–8 weeks of age. (Khan *et al.*, 2006; Muazu *et al*., 2008; Oljira *et al*., 2012)

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 birds in normal flesh, *Eimeria maxima* attacks the middle and lower small intestine. *Eimeria accervulina* attacks mucosal layers of the villi and the sporozoite enter which migrates to the epithelial cell lining, the gland and fund 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 jejunum, 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 (Witlock 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 (Milushev, 1979). *Eimeria acervulina* may parasitize the caeca when large numbers of sporozoites are directly introduced into the caeca. Both schizont and gametocyte develops blit 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 environment. 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). Chickens become infected with *Eimeria* spp. by ingesting infective oocysts (eggs) from litter, soil and contaminated feed and water. The infected birds excrete oocysts into their faeces and are a source of infection for other birds. As *Eimeria* spp. can survive for long periods in infected birds and the environment **(**Khan and Nasir, 2006). The oocysts in faeces become infective through the process of sporulation in about two days (Jeurissen *et al.,* 1996)

2.4.7 Morbidity and Mortality Rates

The mortality rate due to caecal coccidiosis is the highest among coccidiosis (Seneviranta, 1969). Coocidiosis had been reported to result in higher mortality (51.38%); (Demir, 1992) and economic losses ($35 to $200 million/year in USA; Hofstad *et al.*, 1978). 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 birds 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) percents birds at Panjab Agricultural University, India (Sen *et al*., 1981).

2.4.8 Risk Factors

The severity of an infection depends on; the age of birds, *Eimeria* species, number of sporulated oocysts ingested, immune status of the flock and environmental management. Birds 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 birds 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. Birds introduced to an infected building will quickly become infected. Examined risk factors on layer and broiler farms are found with poor hygiene related to personnel, feeding and drinking. 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). Chicks mortality occurs in coccidiosis reveals four major physiological stresses before death: (1) Hypothermia (2) Depletion of carbohydrate stores (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 excysted 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 bird 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 birds. To study the link between numbers of oocysts excreted by infected birds and transmission of *Eimeria acervulina*, experiments were carried out with 42 pairs of broiler chickens using inoculation doses with 5, 50, 500 or 50,000 sporulated oocysts. In each pair one bird was inoculated and the other bird was contact exposed. All contact birds became infected, which occurred on average within 34 hour after exposure to an inoculated bird. Although a higher inoculation dose resulted in higher oocyst excretion in inoculated and contact infected birds, only small non significant differences in transmission rates between groups were found (Velkers *et al.,* 2010).

2.5 LIFE CYCLE

The Eimeria cycle includes two distinct phases; (a) the internal phase (schizogony and gamogony) in which the parasite multiplies in different parts of the intestinal tract and the oocysts are excreted in the faeces (The part of the intestinal tract and the total duration of the internal phase of he cycle is dependant on species), (b) the external phase (sporogony) during which the oocyst must undergo a final process called sporulation before they are again infective. Sporulation requires warmth (25–30°C), moisture and oxygen (Levine, 1982). *Eimeria* spp. has complex life cycles that include three phases: sporogony,merogony, and gametogony (Long, 1982). Depending on species, the endogenous phase in the intestine (which includes merogony and gametogony) consists of multiple stages of asexual reproduction, also called schizogony, which is followed by sexual differentiation, fertilization, and shedding of unsporulated oocysts (Lal *et al*., 2009). The exogenous phase (sporogony) occurs in the environment, where excreted oocysts are stable and eventually sporulate to become infective (Lal *et al*., 2009). The infective oocyst is stable in the environment for several months due to its thick wall, making eradication

of the parasite with disinfectant nearly impossible (Shirley, 1993). The oocysts contain a diploid single cell called a sporont, which undergoes a reduction division in the presence of oxygen which allows it to throw off its polar body and begin sporogeny (Levine, 1982). Infection begins after the mature oocyst is ingested and excysts in response to conditions in the host (Levine, 1982). In the gizzard, mechanical grinding releases the sporocysts into the lumen. Then, bile and trypsin stimulate the release of the sporozoites from the sporocysts via the operculum into the lumen of the duodenum (Levine, 1982). The sporozoite is the infective stage of the parasite and after release from the sporocysts they move to the base of the intestinal epithelial cells lining the villi, where the sporozoite will use proteolytic enzymes to penetrate the host cell. Sporozoites are first observed in intraepithelial lymphocytes (IELs) and then develop inside epithelial cells 7 because host IELs have been shown to transport the sporozoites from the villi to the intestinal crypts (Fernando *et al*., 1987; Trout and Lillehoj, 1996). While in these cells, the sporozoite develops into a rounded body called a first generation trophozoite, then it grows into a first generation schizont, the asexually reproducing stage of the parasite. *Eimeria brunetti* and *E*. *praecox* undergo the entire endogenous phase (both merogony and gametogony) in these villus enterocytes while other *Eimeria* species develop in enterocytes located in crypts before infecting superficial enterocytes during successive stages of shizogony (Shirley *et al.,* 2005). The first generation schizont divides into many first generation merozoites. Merogony beings when one sporozoite releases approximately 1,000 first generation merozoites into the gut lumen, a cycle which repeats 2-4 generations depending on species (Yun *et al*., 2000).

This rupture of intestinal epithelial cells creates extensive cell damage and inflammation in the host and is the basis for the pathologic signs of coccidiosis (Yun *et al*., 2000). Once in the lumen, merozoites penetrate other epithelial cells and develop into second generation trophozoites, which develop into second generation schizonts. The new and numerous schizonts release second generation merozoites which invade new epithelial cells. Each new generation of schizonts results in the

production of more merozoites leading to widespread infection. Gametogony occurs when merozoites develop into either microgamonts or macrogamonts and form a zygote encased by a thick wall that maintains the viability of the oocyst in harsh external environments (Yun *et al*., 2000). Once outside the host, oocysts remain viable in the environment for long periods of time before being ingested and 8 starting the life cycle again (Yun *et al*., 2000). Though gametogony can induce partial immunity, the early endogenous stages are considered the most immunogenic (Shirley *et al*., 2005). Currently, there are eight species of *Eimeria* that parasitize chickens: *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. mivati*, *E. necatrix*, *E. praecox*, and *E. tenella*; however, each species differs in its pathology and immunogenicity (Chapman, 2000; Conway and McKenzie, 2007). The *Eimeria* life cycle contributes to the complexities of host immunity to the parasite, which involves innate and acquired immune systems (Lillehoj, 1998).

The life-cycle is short and involves the ingestion by the bird of sporulated oocysts. Mechanical and chemical factors in the gut then result in the release of sporocytes and sporozoites in the duodenum. The latter invade the duodenal mucosa before undergoing phases of growth and multiplication with periodic release of merozoites into the gut. Merozoites develop within the duodenal cells as gametes, in the form of both macro and microgametocytes, with the latter producing microgametes that migrate to the macrogametocytes. These develop into a zygote and then an oocyst. The life-cycle is rapid, approximately 4-5 days and involves massive multiplication from ingestion of a single infective oocyst. Infected birds pass oocysts in faeces. Oocysts passed in faeces require warm, moist conditions to undergo sporulation, massive multiplication and become infective. Both infected and recovered birds shed oocysts. Under conditions of 25-30^{0}C, this takes 1-2 days. Sporulated oocysts have the ability to survive outside the host for very long periods. Since sporulation does not occur below 12^{0}C or above 39^{0}C, during winter months spores may remain dormant. Sporulation can continue when temperatures increase, although prolonged periods at low temperatures can destroy the viability of the oocytes. This may be an important factor for outdoor poultry systems (Fanatico, 2006).

2.6 PATHOGENECITY

Pathogenecity is related to the dose of infective oocysts received by the bird 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 birds 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 colonisation by other harmful bacteria, such as *Clostridium perfringens*, which causes necrotic enteritis (Immerseel *et al*., 2004). The species important in broiler production include *E. tenella* (90%), *E. maxima*, *E. acervulina*, and *E. mivati*, the species important in breeder and layers are *E. burnetti* and *E. necatrix*. Seven species infect turkeys, the big three of concern are *E. meleagrimitis*, *E. adenoeides* and *E. gallapovonis (*Julie D., 1999)

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 4^{th} and 5^{th} days aggregation of these schizonts appear as small whitish opacities. Later punctate hemorrhage appear in the centre of the whitish areas. The unopened intestine thus presents a spotted appearence, 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 profuae 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 (Van Dor Nick and Becker, 1957). Eimeria oocysts are broadly ovoid, smooth and without micropyle. There are three asexual generation of merozoite. *E. necatrix* is also a common species. Its first and 2nd generation merozoite occurs 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. acervulina* 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' merozoite 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 asxual generation of merozoites. sporozoites 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 birds tend to huddle together, have ruffled feathers and show signs of depression which range from decreased growth rate to a high percentage of visibly sick birds, severe diarrhea, and high mortality. Feed and water consumption are depressed. Weight loss, development of culls, decreased egg production, and increased mortality may accompany outbreaks (Biggs P., 1982). Mild infections of intestinal species, which would otherwise be classed as subclinical, may cause depigmentation and potentially lead to secondary infection, particularly *Clostridium* spp infection. Survivors of severe infections recover in 10–14 days but may never recover lost performance (Richard W. Gerhold, Jr., 2014).

The lesions are almost entirely in the intestinal tract and often have a distinctive

location and appearance that is useful in diagnosis. The birds 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* (Arakawa *et al*., 1981; Baba *et al*., 1982; Helmbolt and Bryant, 1971). Infestation with *E. tenella* also increases the severity of *Histomonas meleagridis* infection in chickens (McDougald and HU, 2001). Less virulent strains will result in poor growth and reduced feed efficiency. Hence the losses resulting from coccidiosis may be variable. There is normally a reduction in feed and water intake (Williams, 1996). Death occurs in chicken mostly due to hemorrhage caused by large second generation schizonts stage of the *Eimeria* (Waxier, 1941). Reduction of feed and water intake takes place in the experimentally induced coccidiosis in chickens (Reid and Pitoais, 1965). Coccidiosis is generally acute in onset and is characterized by depression, ruffled plumage, and diarrhea. Birds infected with *E. tenella* show pallor of the comb and wattles and blood-stained caecal droppings (Simon M., 2005). 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 hemorrhagic enteritis and even death in young birds (Levine, 1983). *Eimeria tenella* as the most pathogenic of all the avian coccidia. It causes cecal hemorrhage after a moderate or severe infection and death occurs mostly on 5^{th} or 6th day after infection (Tyzzer, 1929)

2.8 PATHOLOGY

2.8.1 Macroscopic Lesions

In moderate infections there is a thickening of the gut wall, a pinkish or blood-tinged catarrhal exudates 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, 1942). 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 haemorrhagic 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, 1955). There are two types of coccidiosis hemorrhagic and catarrhal. In hemorrhagic type, lesions are distension of caeca with blood, blood clots and reddish brown contents whereas in catarhal type, petechial spots seen throughout the serosa associated with watery ingesta mixed with mucus. Due to *Eimeria necatrix*, the middle part of the small intestineis 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. Greyish white pin point foci in the mucosa occurs in the earlier part of the small intestine. Intestinal contents are liquid and mixed with mucous. Streaky hemorrhage 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 hyperaemic with occational pin point haemorrhage. Sharp lines of demarcation between affected and unaffected areas are noticed. The changes occur due to mixed infection are distention of entire length of small intestine along with crimson appearance, haemorrhagic spots and greyish white foci seen throughout the serosa. The intestinal contents are reddish brown in colour with blood clots and fibrins threads. Large masses of fibrin clots with blood streaks 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, 1967). The lumen is filled with blood and pieces of loosened ulcerated mucosa. 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 pinpoint lesions 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 lnfection causes coalesent 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 occurs in extremely heavy infection. The lesions in the lower sma1l 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 (Reid, 1972). The enlargement of caeca and small areas of hemorrhage. By 4th day, caeca is enlarged to three times of normal size, spotted irregular focal heroorrhagic areas appear on the serosal surface (Reid, 1972).

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 over all 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.

Eimeria tenella is the cause of so called caecal or bloody coccidiosis of chicks. Involvement of the caeca 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 are 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, visible through the serous surface 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 and proliferation of lymphoid tissue in the lower small intestine (Novilla *et al*., 1972). The histopathological changes occurs due to *E. tenella* are desquamation of epithelium, enlargement of internal glands and developmental stages of parasite and cellular infiltration are also reported. 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 hemorrhage around the enlarged epithelial cells, infiltration of macrophages and lymphocytes, secretory vacuolation of glands and developmental stages of parasite is noticed almost in the entire thickness of mucosa (Jagadeesh *et al.,* 1976).

2.9 IMMUNITY

Eimeria reside outside the host for part of their life cycle but, the majority of it is completed inside the host during asexual and sexual stages of development occurring

inside or outside enteric tissues. Once the bird ingests the viable oocyst(s), a cascade of events occurs involving both non-specific and specific defense mechanisms of immunity (Lillehoj and Lillehoj, 2000). It is to be expected that the mechanisms responsible for immunity are complex due to the complexity of the parasite life cycle. Despite all of the research completed on immunity to *Eimeria*, no clear picture has emerged as to how complete resistance is acquired and which mechanisms are sequentially involved in generation of immunity (Rose *et al*.,1979; Danforth and Augustine, 1989).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; 1994b; Wallach *et al.*, 1995). Birds of all ages are susceptible. Although the risk of coccidial infection may increase with age (Rose, 1967; Hein, 1968), the effects of infection may be more serious in chicks. 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 birds can be more susceptible to coccidiosis (Biggs *et al.*, 1968). Addition of coccidiostates in the ration had been one of the best options for the control of coccidiosis; however, egg laying birds are given coccidiosate-free ration during the egg laying period and an outbreak of coccidiosis at that stage will not only result in massive death casualties, but it could lower egg production performance of the birds. The pullet should therefore, have complete immunity against coccidiosis before initiation of egg lay (North, 1984).

2.10 CONCURRENT INFECTIONS OCCURRING DURING THE COURSE OF COCCIDIOSIS

Coccidiosis are 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 *Closrtidium 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 coccidial 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

Coccidiosis is one of the most important and common diseases that affect poultry, it results in a great economic loss all over the world **(***Nematollahi et al.,* 2009). Losses due to avian coccidiosis had been estimated 1-40 million dollar 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 (Zimmermann, 1957). The economic importance of the disease is due to its high rate of morbidity and mortality in young birds, reduced feed conversion efficiency and egg production in sub-clinical cases (Adhikari *et al.*, 2008). The economic loss to poultry industry has been estimated considering the major economic parameters. The estimation has revealed that commercial broiler 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 broiler industry of India. The overall comparison of economic traits for all the types of poultry sector it has shown that reduced body wt gain and increased FCR are the major parameters from which 68.08 per cent and 22.70 per cent 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).

2.12. TREATMENT AND CONTROL

More than 50 years anticoccidial feed additives have been used to prevent or treat coccidiosis in poultry. Anticoccidials can be classified as follows (Jeffers, 1997; Chapman, 1997; Allen and Fetterer, 2002). Anticoccidial drugs added to the feed are a good preventive measure and are well adapted to large-scale use, but prolonged use of these drugs leads inevitably to the emergence of *Eimeria* strains that are resistant to all anticoccidial drugs, including ionophores (Ruff and Danforth, 1966; Chapman, 1994; 1997; 1998; Allen and Fetterer, 2002)

Chemicals: These compounds are produced by chemical synthesis and have a specific mode of action against parasite metabolism, such as amprolium, nicarbazin and diclazuril.

Polyether ionophores: They are produced by fermentation of Streptomyces or Actinomadura and they are the most commonly used agents, such as salinomycin, monensin, lasalocid and narasin. They act through a general mechanism of altering ion transport and disrupting osmotic balance in the parasite.

Vaccines

Vaccines are one of the most valuable public health tools that have been developed by man (Payette and Davis, 2001). The development of resistance of coccidia to anticoccidial drugs (Chapman, 1997, Williams, 2002), Drug resistance to anticoccidial drugs is described worldwide to all coccidiostats and to all *Eimeria* species(Zhang *et al*., 2013).

Poultry House Management

The high standard of flock hygiene, sanitation and poultry farm management helps in achieving optimal benefit from the anticoccidial drugs in preventing coccidiosis (Chapman, 1997).

Alternatives for anticoccidial drugs

The extensive use of the anticoccidial drugs for prevention and control of coccidiosis in poultry has been a major factor in the success of the industry. This beneficial use of anticoccidial drugs is associated with a widespread drug resistance of coccidia in the United States, South America and Europe (Jeffers, 1974a; 1974b; Litjens, 1986; McDougald *et al*., 1986; 1987; McDougald, 2003).

CHAPTER III

MATERIALS AND METHODS

3.1 EXPERIMENTAL CHICKENS AND RESEARCH AREA

The chickens of different commercial poultry farms (both layer and broiler) were considered as experimental birds. Coccidia outbreaks in different Upazilla were investigated to find out diseased and dead birds. The experiment was carried out in the Department of Pathology and Parasitology (PPS), Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. Representative samples of diseased and dead birds (intestinal part like dudenum, jejunum, caecum) from each upazilla were collected randomly from the natural case of infection at Dinajpur District.

A total of 12 farms (both layer and broiler) were visited, among which 354 diseased and dead birds were examined. But 31(19 broilers and 12 layers) birds were found to be positive for coccidiosis. The number of birds in the farms was variable ranging from 250 to 3500 and they were reared on litter. A detail flock history in relation to the incidence of disease including housing system, location of poultry farms, sources of birds, age and population of the birds per flock, rearing system, litter material, feeding and watering system, bio-security 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 birds 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 during the physical visit of farms.

3.2 RESEARCH PERIOD

The duration of experiment was 6 months from, January to June, 2014.

3.3 MATERIALS

3.3.1 SAMPLING LOCATION

Sources of the population in this study were raised commercially by the farmers from different upazilla at Dinajpur district. From the selected area, all the dead as well as diseased birds were collected for further examination. Coccidiosis affected birds were collected, examined and send to the laboratory for detailed necropsy and histopathological examination.

3.3.2 INSTRUMENT AND APPLIANCES

Equipment and appliances for necropsy:

- Scissors
- Forceps
- Gloves
- Musk
- Scalpel
- Knife
- A pair of shears,
- 10% neutral buffered formalin

Equipment and appliances for histopathology:

- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene
- Hematoxylin and Eosin stain

- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX)
- Microscope

Equipment and appliances for parasitological examination of faeces

a) Direct smear technique-

- ❖ Beakers
- ❖ Stirring rod
- ❖ Test tubes
- ❖ Microscope
- ❖ Slides
- ❖ Cover slips

b) Floatation technique-

- ❖ Beakers
- ❖ A tea strainer
- ❖ Stirring rod
- ❖ Test tubes
- ❖ Microscope
- ❖ Slides
- ❖ Cover slips
- ❖ Flotation fluid

3.3.3 CLEANING AND STERILIZATION OF REQUIRED GLASSWARE

Test tubes, glass tubes, glass slides, cover slips, beakers, pipettes, reagent bottles, glass bottle, spirit lamp, measuring cylinders etc. were used in this study. The conical flask, measuring cylinder, beakers, glass slides, cover slip, were prepared for histopathological study. 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 dishwashing detergent solution, the glassware were cleaned by brushing and washed thoroughly in running tap water and rinsed three times in distilled water. The cleaned glasswares were then dried on a bench at room temperature.

3.3.4 CHEMICAL AND REAGENTS USED

10% neutral buffered formalin, Xylene, Hematoxylin and Eosin stain, PBS, Distilled water etc were used for necropsy and histopathology of collected samples.

3.3.4.1 PREPARATION OF HARRIS' HEMATOXYLIN SOLUTION

Hematoxylin crystals	5.0g
Alcohol (100%)	50.0 ml
Ammonium or potassium alum	100 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.

3.3.4.2 PREPARATION OF EOSIN SOLUTION

1% stock alcoholic eosin

Eosin Y, water soluble	1 g
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	1part
Alcohol, 80%	3 parts

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

3.4 METHODS

3.4.1 THE MAJOR WORKS OF THE PRESENT STUDY

- Clinical Examination of birds.
- Fecal and intestinal swab examination for oocysts determination.
- Necropsy examination of visceral organs to detect lesions of coccidiosis in suspected dead and diseased birds.
- Histopathological examination of caecum, colon, duodenum and jejunum.

3.4.2 EXPERIMENTAL LAYOUT

Dead and diseased birds (layer and broiler) were collected from different farm at Dinajpur district

↓

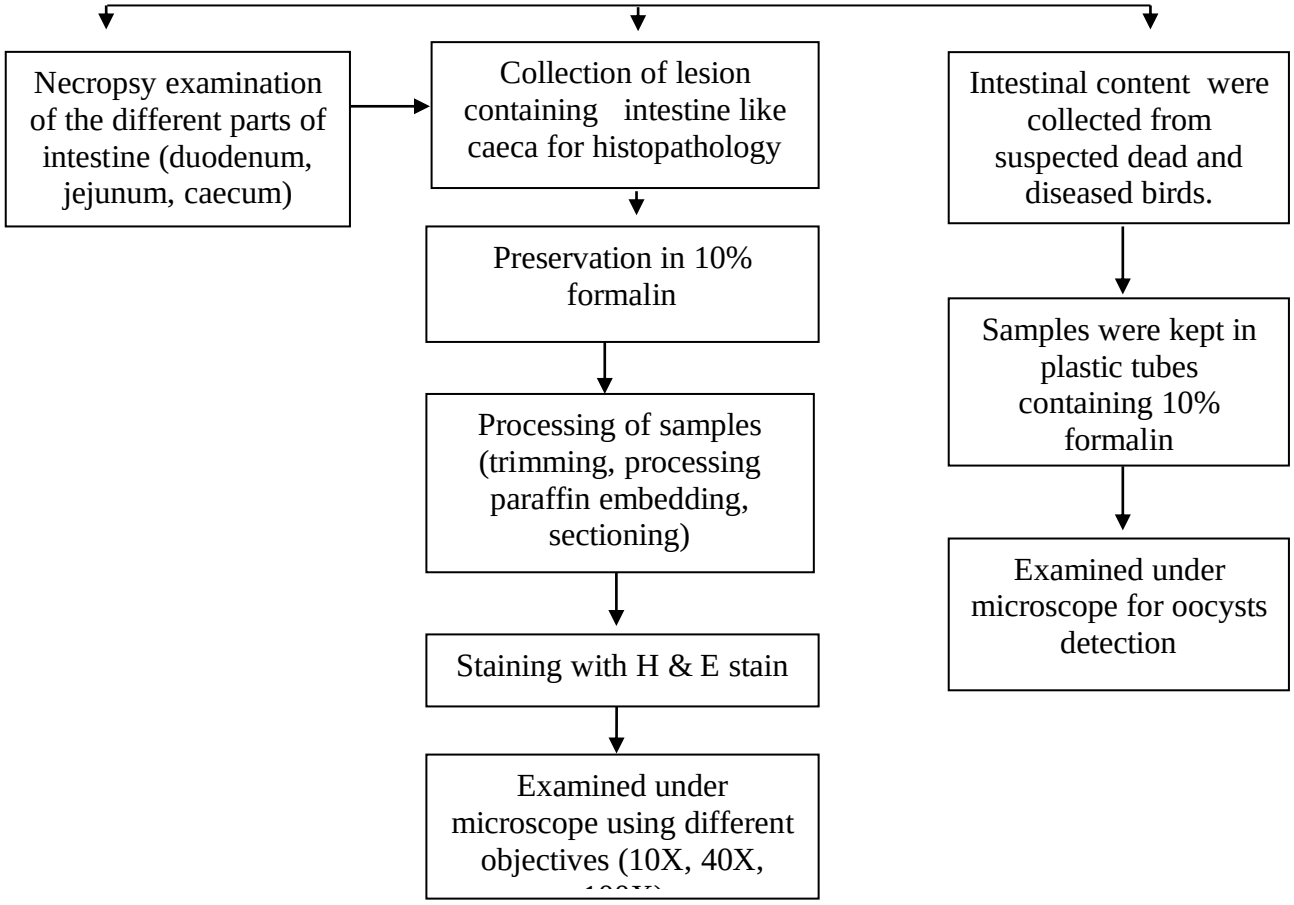

Fig. 2 Schematic illustration of the experimental layout

3.4.3 FIELD INVESTIGATION OF OUTBREAKS AND COLLECTION OF SAMPLES

At the time of field investigation a details of history, age, incidence, morbidity rate, mortality rate, vaccination status were recorded. Clinical signs were observed and postmortem examinations were done on dead and clinically affected birds. Samples were collected from five broiler and three layer birds from six different Upazillas (Dinajpur sadar, Chirirbandar, Parbotipur, Fulbari, Birampur and Nawabgonj) at Dinajpur district. Intestinal organ like duodenum, jejunum,caeca were collected during necropsy for further study. All the diagnostic works were carried under the Laboratory of Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU). Clinical diagnosis and in some cases necropsy examinations were carried out at the place of sampling where as histopathology of all samples were done in the laboratory.

3.4.4 CLINICAL EXAMINATION

The general health condition and age of the chicken were recorded. The chicken were observed to detect clinical signs. The clinical signs were observed from the visual examination. The clinical signs were recorded during the physical visit of the affected flocks and the farmer's complaints about the affected birds were also considered.

3.4.5 NECROPSY FINDINGS OF SUSPECTED BIRDS

The necropsy was done on the suspected dead and diseased birds taken from different upazilla of Dinajpur district. At necropsy, gross tissue changes were observed and recorded carefully by systemic dissection. The samples were also collected in 10% neutral buffered formalin for the histopathological study. The routine necropsy examination was carried out as follows-

- 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 bird 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 bird was positioned in such way so that the legs 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 for detection of anaemia.
- Body cavity of bird 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 cotents and mucosal surfaces gradually.
- The caecal junction and the caecum at either side were opened and were examined in similar manner.

Gross lesion

Gross morbid lesions of different organs were observed after necropsy examination of the birds.

3.4.6 HISTOPATHOLOGICAL EXAMINATION

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

3.4.6.1 PROCESSING OF TISSUES AND SECTIONING

The tissues were processed and sectioned as followed:

Collection of tissue and Processing: During tissue collection the following point were taken into consideration.

The tissues were collected in conditions as fresh as possible. Normal and diseased tissues were collected side by side. The thickness of the tissues was as less as possible (5mm approximately). The tissues (intestinal part like caecaum, duodenum, jejunum) were collected from the birds that were examined in Dinajpur area. The representative tissues with its normal periphery were- collected.

Fixation: 10% formalin was taken in the plastic container. (10 folds of the tissue size and weight) and fixed tissue for 3-5 days.

Washing: The tissues were trimmed into a thin section and washed over night in running tape water to remove formalin.

Dehydration: The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule. The tissues were dehydrated in 50%, 70%, 80%, 95%, 100%, 100%, and 100% ethanol, one hour in each.

Impregnation: Impregnation was done in melted paraffin at 56- 60°c for 3 hours.

Sectioning: Then the tissues were sectioned with a microtome at 5-µm thickness. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The sections were allowed to spread on warn water bath at 40-42°C. Then the sections were taken on grease free clear slides.

Drying: The slides containing section were air dried and kept in cool place until staining.

3.4.6.2 ROUTINE HEMATOXYLIN AND EOSIN STAINING PROCEDURE

The sectioned tissues were stained as described be

- The sectioned tissues were deparaffinized in three changes of xylene (three minutes in each).
- Then the sectioned tissues were rehydrated through descending grades of alcohol (three changes in absulate alcohol, three minute in each, 95% alcohol for two minutes, 80% alcohol for two minute, 70% alcohol for two minutes) followed by distilled water for 5 minutes.
- The tissues were stained with hematoxylin for fifteen minutes and washed in running tap water for 10-15 minutes.
- Then the tissues were differentiated in acid alcohol by 2 to 3 quick dips (1 part HCI and 99 parts 70% alcohol) and washed in tap water for five minutes followed by 2-3 dips in ammonia water until sections were bright blue.
- Then the section on the slide were stained with eosin for one minute.
- The section was differentiated and dehydrated in alcohol (95% alcohol: three changes, 2-3 dips each, absulate alcohol: three changes 2-3 minute for each cleaned in zylene three changes, five minute in each).
- Tissues were mounted with cover slip by using DPX.
- The slide were dried at room temperature and examined under a low (10X) and high (40X, 100X) power microscopic field.
- Then the images of the stained section were taken by digital camera (Sony 14.2 Mega pixel).

3.4.7 PARASITOLOGICAL EXAMINATION OF FAECES

3.4.7.1 Collection of faeces

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

3.4.7.2 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 add 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 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.
- Then floatation fluid was added into the container which containing feces.
- The feces were mixed thoroughly with the flotation fluid with stirring device.
- Then the fecal 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 fecal 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 slides.

- ❖ The slides were examined under microscope for detection oocysts in low (10x) and high magnification (40x, 60x and 100x).

3.5 STATISTICAL METHODS

3.5.1 DETERMINATION OF PREVALEANCE

Prevalence of a disease is the proportion in a given population which have a particular disease at a specified point in time, or over a specified period of time. In this study the prevalence was calculated by the following statistical formula-

$$\text{Prevalence } (\%) = \frac{\text{Coccidia infected birds during specified time period}}{\text{Birds population during the same time period}} \times 100$$

3.5.2 DETERMINATION OF MORTALITY RATE

Mortality rate is a measure of the number of deaths due to a specific cause in a given population. In this study the mortality rate was calculated by the following statistical formula-

$$\text{Mortality rate } (\%) = \frac{\text{Deaths occurring during a given time period}}{\text{Birds population during the same time period}} \times 100$$

CHAPTER IV

RESULTS

Different upazila like Sadar, Chirirbandar, Parbotipur, Fulbari, Brampur, and Nawabgonj of Dinajpur district were considered as the study population for this research work. The dead and diseased birds were collected and subjected to pathology laboratory of Hajee Mohammad Danesh Science and Technology University (HSTU) to determine the prevalence, mortality, gross and histopathological lesion of Coccidiosis in birds of Dinajpur district. The results of different clinical and pathological examination are as follows.

4.1. Clinical examination

The general health condition and age of the birds were recorded. The present clinical examination identified the different type of clinical signs caused by different species of *Eimeria*. During clinical examination following clinical signs were depression and ruffled feather along with paler comb and wattle (Fig 3), attachment of feaces around the vent (Fig 4), blood mixed feaces (Fig 5) and dehydrated anaemic carcass (Fig 6) .Sometimes drooping wings, less egg production, Weight loss were also found during field examination. Bloody diarrhea was considered to be a most important clinical sign.

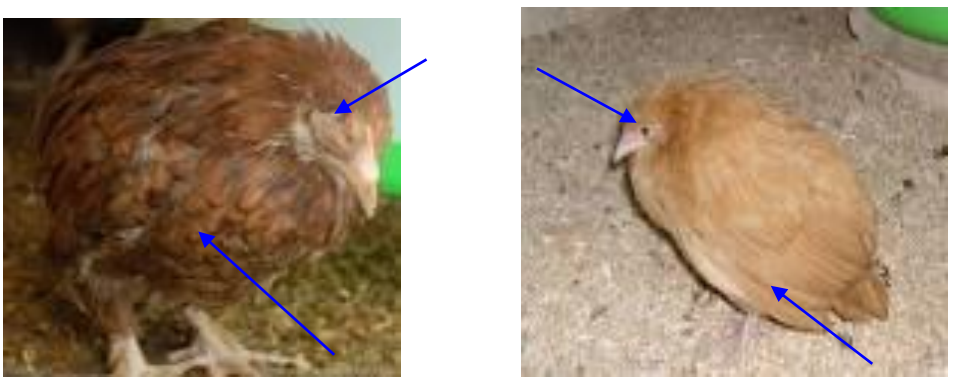

Fig. 3 Birds showing depression and ruffled feather along with paler comb and wattle

Fig. 4 Attachment of feces around the vent

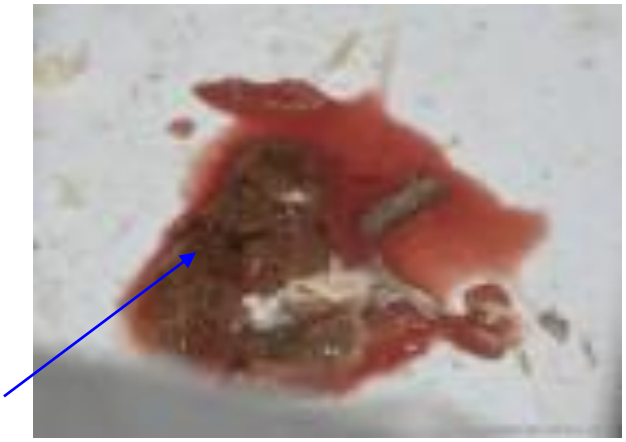

Fig. 5 Bloody feaces

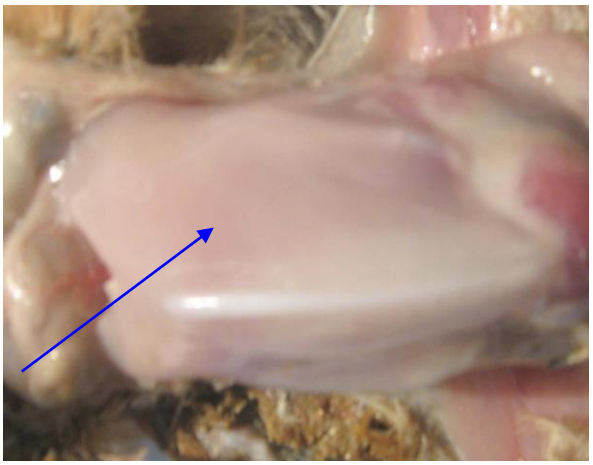

Fig. 6 Dehydrated and anaemic carcass

4.2 Degree of Infectivity

The study revealed the following status of prevalence of Avain coccidiosis (AC). The overall prevalence at Dinajpur district in Broiler is 9.65% and whereas in Layer 7.10%. The highest and lowest prevalence was observed both broiler and layer at Parbatipur upazila 15.56% and 4.00% respectively and Sadar upazila shows highest prevalence 12.50% in layer whereas lowest in broiler at Birampur upazila (5.55%) (Table-1and 2). Proportional mortality rate of coccidiosis in different age group is shown in table-3 where 0-4weeks of birds show highest mortality rate 10.66% and lowest 2.1% at >8 weeks of age. Graphical presentation of prevalence at different upazila in Dinajpur district (Fig. 7) and mortality rate of AC in different age group is shown in (Fig. 8)

Table 1 Prevalence of Coccidiosis in Broiler and Layer at different Upazilla in Dinajpur district

Name of Upazilla	Type of Birds	No.of necropsy done	No. of infected birds	Percentage (%)
Sadar	Broiler	30	3	10.00
	Layer	24	3	12.5
Chrirbandar	Broiler	21	2	9.52
	Layer	17	1	5.88
Parbatipur	Broiler	45	7	15.56
	Layer	25	1	4.00
Fulbari	Broiler	49	4	8.16
	Layer	34	2	5.88
Birampur	Broiler	18	1	5.55
	Layer	39	3	7.69
Nawabgonj	Broiler	22	2	9.09
	Layer	30	2	6.67
Total		**354**	**31**	

Table 2 Prevalence of different upazilas adjusted by DMRT

Upazila	Prevalence (%)	
	Broiler	Layer
Sadar	10.00 [b]	12.50 [a]
Chirirbandar	9.520 [b]	5.888 [bc]
Parbotipur	15.56 [a]	4.000 [c]
Fulbari	8.160 [bc]	5.880 [bc]
Birampur	5.550 [c]	7.690 [b]
Nawabgonj	9.090 [b]	6.670 [b]
Mean±SE. Mean	9.65±0.79	7.10±0.69
LSD	NS	NS
CV %	16.39	17.24

NS= No level of significance

LSD = Least Standard Deviation

CV = Co-variance

SEM = Standard Error Mean

Table 2 showed highest prevalence by the symbol of [a] and [c] possed lowest prevalence. The value of co-efficient of variation indicates they are more homogenous

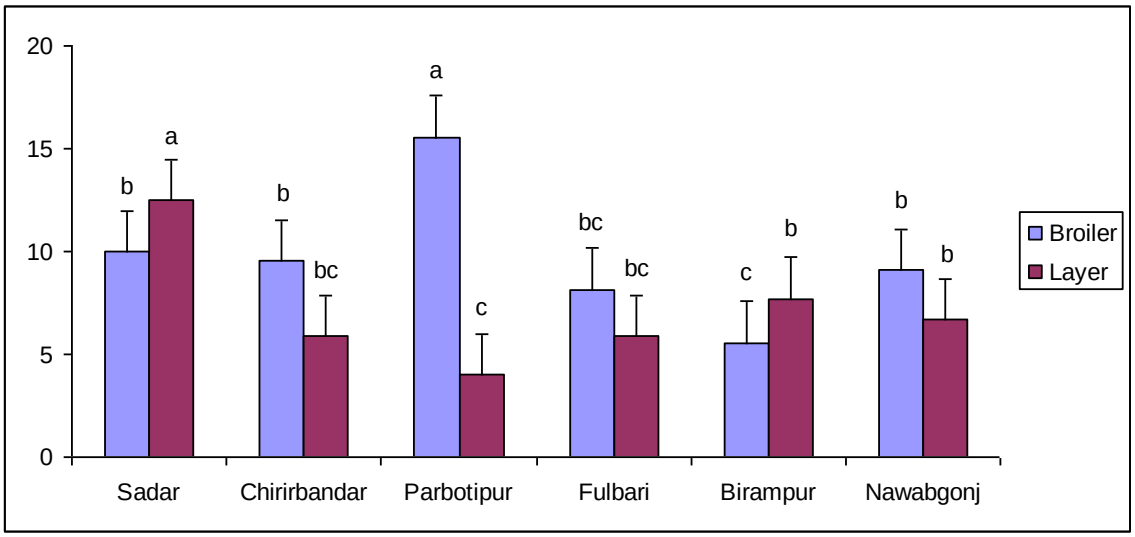

Fig. 7 Prevalence of coccidiosis at different upazilla of Dinajpur district. Each bar represents Mean ± SEM value

Table 3 Mortality rate of coccidiosis in different age group

Age of Birds(Weeks)	No. of birds observed	No. of dead birds	Percentage (%)
0-4th	150	16	10.66
5th-6th	75	7	9.33
7th-8th	81	5	6.17
>8th	48	1	2.1

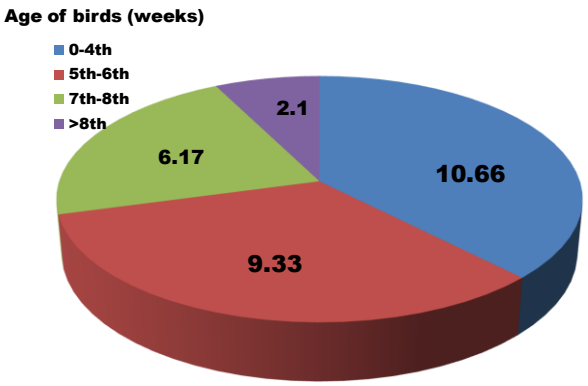

Fig. 8 Mortality rate of Coccidiosis in different age group

4.3 Necropsy examination

Necropsy findings in different intestinal regions of chicken were detected by postmortem examination (Fig. 9) and the findings were enlargement and ballowing shape of caeca with profuse clotted blood (Fig. 10). Reddish brown and blood clotted intestinal contents were found in the lumen of caeca (Fig. 11). Pin point hemorrhage on intestinal mucosa (Fig. 12).

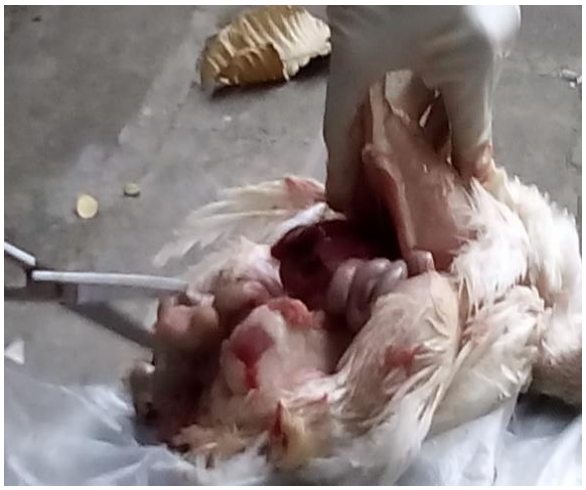

Fig. 9 Postmortem examination of bird

A B

Fig.10 A. Normal caeca.

B. Enlargement and ballowing shape of caeca with profuse clotted blood

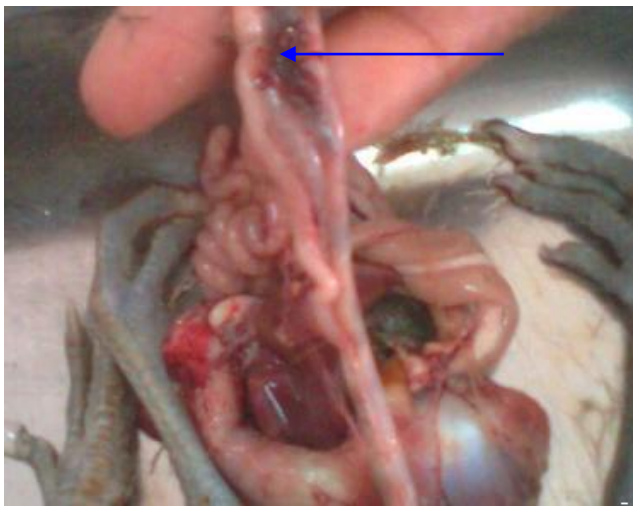

Fig. 11 Blood clotted intestinal contents found in the intestinal lumen of caecum

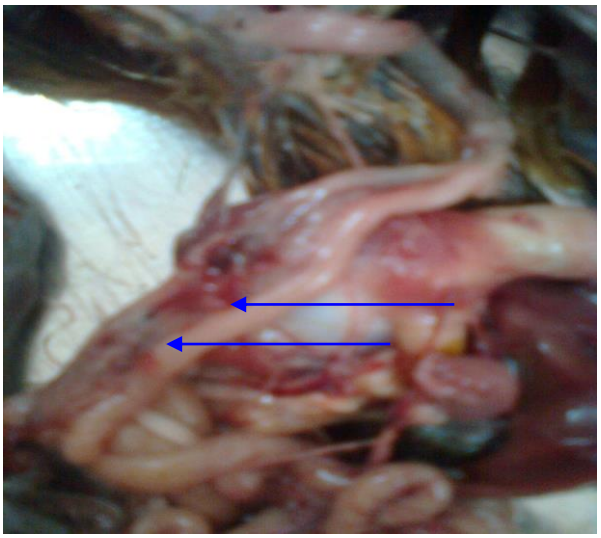

Fig. 12 Pinpoint haemorrhage of intestinal mucosa

4.4. Histopathological study

In present study, distortion of normal architecture of intestine with desquamation of lining epithelia (Fig. 13). The villi of the mucosa were destroyed and disorganised and there was no continuation in the lining epithelial cells of villi (Fig: 14) Degeneration of epithelial cells, glands, intestinal villi and infiltration of inflammatory cell in the musculature were also present (Fig. 15). Necrosis and hemorrhage were found around the invading gland, epithelial cells and other structure (Fig. 16)

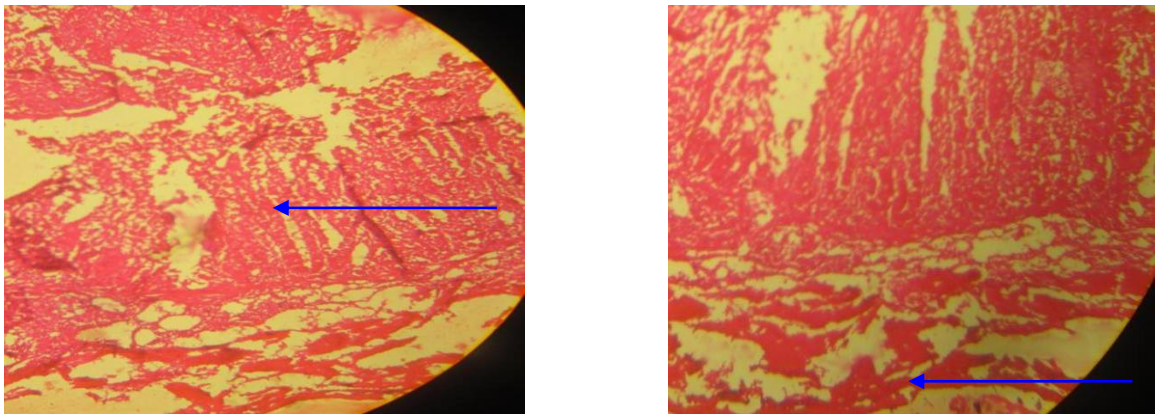

Fig. 13 Distortion of normal architecture of intestine and desquamation of lining epithelia

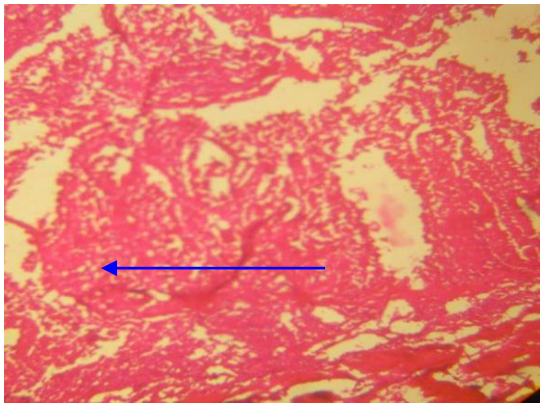

Fig. 14 The villi of the mucosa were destroyed and disorganised and there was no continuation in the lining epithelial cells of villi

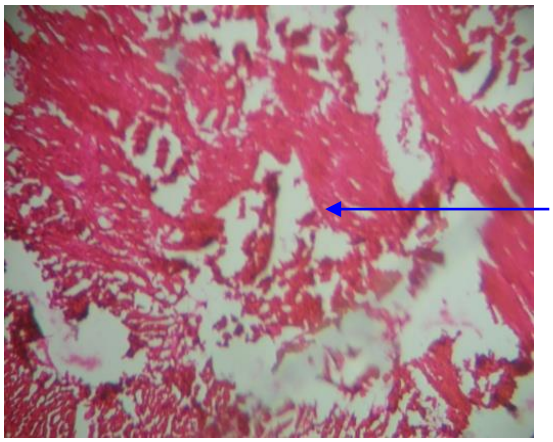

Fig. 15 Degeneration of epithelial cells, glands, intestinal villi and infiltration of inflammatory cell in the musculature

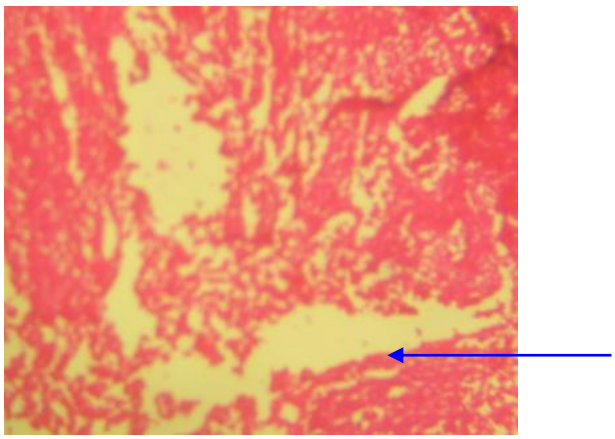

Fig. 16 Necrosis and haemorrhage present around the epithelial cell

4.5. Parasitic examination

In this study, the oocyst of cocccidia (*Eimeria* sp.) were detected in the faeces (Fig. 17)

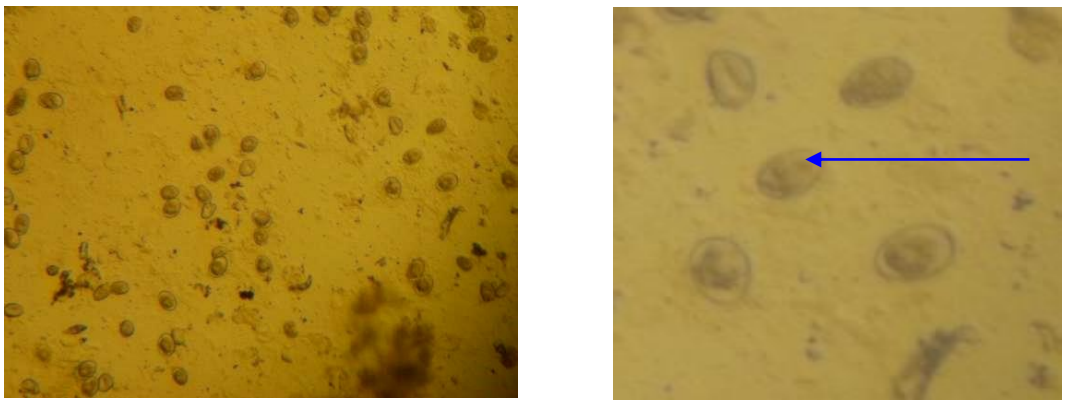

Fig. 17 Oocyst of *Eimeria* sp. in feaces

CHAPTER V

DISCUSSION

The present study was carried out to investigate the prevalence and pathology of avian coccidiosis in birds at commercial farms (layer and broiler) in different upazilla of Dinajpur district from January to June, 2014. A total of 354 diseased and death birds of 12 farms were examined on the basis of history, clinical sign, gross and microscopic lesions.

5.1. Prevalence

Coccidiosis is the most prevalent intestinal parasitic disease of poultry through out world. So the study was conducted in different farms at different upazilla in Dinajpur district. Total 354 diseased and dead birds were randomly examined out of which 31(19 broiler and 12 layer) birds found to be positive for coccidiosis among 12 farms. Different species of *Eimeria* were found to be prevailed in those farms. There was a relationship found among the prevalence of coccidiosis at different upazilla in Dinajpur district. The prevalence was maximum in Parbatipur Upazilla (15.56%) in Broiler, whereas in Layer Sadar Upazilla show high (12.50%) (table-2) and minimum prevalence was found (5.55%) in Broiler at Birampur; but in layer (4.00%) found in parbatipur (table-2). So the prevalence mean difference in Broiler is high that is (9.65%) from Layer (7.1%) which is low because of there self limiting character. This observation more or less similar to those authors report where the prevalence of coccidiosis have been reported from Bangladesh by Bhattacharjee *et al*., (1996); Talha *et al.,* (2001) and Giasuddin, *et al* (2003) who reported (9.40%, 5.51%a, 9.17% respectively). In West Bangle 85 (10.91%) cases of coccidian is recorded by Bhattacharya Pramanik, (1987) and give the proof of the endemicity of coccidiosis in this rearing system. This relatively higher prevalence of coccidiosis could be ascribed to the confinement and deep litter-based rearing system compared to caged birds.

Young birds are more susceptible and more rapidely display signs of disease, whereas older birds are relatively resistant. Chicks are not fully immunized and can experience great mortality in coccidiosis outbreak Chapman *et al*., (2005). Typically, the disease is seen in birds of 3-6weeks old, before they have acquire immunity. The proportional mortality rate of coccidiosis in different age group were 10.66%, 9.33%, 6.17% and 2.15% in 0-4weeks, 5-6weeks, 7-8 weeks and above 8 weeks respectively which is similar to the observation by Kamarth (1955); Rose, (1999); Humpphrey, (1973) and Kogut *et al*., (1993). The result also contrast with the observation of Etuk *et al*., (2004) who recorded a higher prevalence of coccidiosis in adult layer birds than in other age categories which is different from this study may be due to location, season, age difference, sex, breed and other managemental factors.

5.2 Clinical examination

Clinical manifestation of chickens naturally infected with coccidiosis was studied. During this study , the common clinical manifestations in the chicks suffering from natural coccidiosis were found as bloody diarrhoea, anaemic carcass (Fig 6), attachment of faeces around the vent (Fig:4), blood mixed feces (Fig:5), Depression and ruffled feather with paler comb and wattle (Fig. 3). These findings are also suppoted by Reid and Pitoais (1965) and Williams (1996) but there may be slight variation due to weather, season and other factors.

Weight loss, reduction in egg production, damp litter and death occurs mostly on 5th or 6th day after infection were also found inthis study. Similar findings were reported by Waxier (1941), Ruff *et al*. (1976) and Tyzzer (1929); but there were a small variation due to management of birds, location and others factor like ventilation, feeding, watering, time of vaccination etc.

5.3. Necropsy examination

A total number of 31 dead and sick birds suspected to be infected with coccidiosis collected from small scale commercial broiler and layer farm in Dinajpur were subjected to postmortem examination. Gross lesion of the various organs of the affected chickens were studied. At necropsy, the major pathological lesions were enlargement and ballowing shape of caecum (Fig. 10) with pin point hemorrhage and reddish brown or blood clotted intestinal contents in the lumen of caeca (Fig. 11) these gross lesion are also reported by Bertke (1955), Reid (1972) and Becker (1959).

Pin point hemorrhage on intestinal mucosa (Fig. 12), hemorrhagic enteritis, mucoid to blood-tinged exudates and profuse mucosal bleeding in the caeca. This observation is similar to those reported by Paul (2009), Jagadeesh *et al*., (1976), Levine, (1983), Arakawa *et al*., (1981); Helmbolt and Bryant, (1971) but some difference were found in this study during postmortem examination of birds like degeneration of internal organ, discolaration of the organ etc.

5.5 Histopathological study

The histopathological change founded in the present study were severely damage mucous membrane, brake down of continuty of mucosal layer of intestine, distortion of architecture and desquamation of lining epithelia (Fig. 13), necrosis and hemorrhage around the invading gland and epithelial cell (Fig. 14), degeneration of epithelial cells, glands, intestinal villi and infiltration of inflammatory cell in the musculature (Fig. 15). The villi of the mucosa were destroyed and disorganized and there was no continuation in the lining epithelium and reactive cell infiltration (Fig. 16). This observation is similar to those reported by Jagadeesh *et al*., (1976), Novilla *et al*. (1972) but some variation may be found due to strain, coloring agent, formalin, and other agent, lab environment etc.

CHAPTER VI

SUMMARY AND CONCLUSION

The present study was conducted to explore prevalence and pathological investigation of avian coccidiosis based on clinical, parasitological, necropsy and histopathological feature. The study was conducted in different poultry farms at different upazilla in Dinajpur district. Total 12 farms were visited among which 354 diseased / dead birds were examined out of which 31 birds (19 broiler and 12 layer) were found to be positive for coccidiosis. Different type of *Eimeria* were found to be prevailed in those poultry farm. Prevalence of coccidiosis was recorded 9.65% in broiler and 7.1% in layer. The overall prevalence of coccidiosis is lower because the farmer are intensely aware of coccidiosis and other parasitic disease now a days. They usually use coccidiostats regularly. Proportional mortality rate of coccidiosis in different age group were 10.66%, 9.33%, 6.17 % and 2.1% in 0-4weeks, 4-6weeks, 6-8weeks and above 8 weeks, respectively.

The clinical signs of the affected birds were more or less similar to the signs generally developed in coccidiosis, and clinically characterized as bloody diarrhea, reduction of feed and water intake, anemia, ruffled feather, depression, drooping wings, pale or anemic carcassand decreased egg production.

At necropsy, enlargement and discoloration of caecum with pin point hemorrhage was observed. Reddish brown and blood clotted intestinal contents were found in the lumen of caeca. There was profuse congestion and pin point hemorrhage on intestinal mucosa.

- Histpathologically**,** the mucous membrane was found to be severely damaged and there was broke down the continuity of mucosal layer of intestine. Distortion of architecture and desquamation of lining epithelia were present. Infiltration of inflammatory cells in the musculature was also present. 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 flocks are lower because of proper management like bio security, ventilation, good litter and good food.
- The farmers are intensely aware of coccidiosis now a days and they usually use coccidiostats and vaccine like livacox®, coxvet®, Coccicure® etc routinely.

On the basis of this study it is assumed that coccidiosis is a problem at poultry industry in Dinajpur district of Bangladesh. So overcome this problem farmers should be followed routine preventive and control measure which is prime essential for substantial improvement in poultry production at Dinajpur.

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