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**Research Article** 



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# Haemato-Pathological Changes Caused by Coccidiosis in Sonali Chicken at Dinajpur Area of Bangladesh

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### <u>Abstract</u>

This study was conducted to investigate the prevalence, pathological status and hematological parameters of avian coccidiosis. A thorough 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 15 farms were visited and 300 diseased birds were taken to perform postmortem. After postmortem 46 birds were found affected with coccidiosis. Blood sample were collected from few infected birds and tested for hematological value. Coccidiosis caused by *Eimeria sp.* instigated a declining (mean  $\pm$  SD) in RBC  $(1.9\pm 0.3)$  and PCV  $(23.5\pm 4.8)$ . The differential WBC (leukocyte) count showed an increase in lymphocytes  $(64.3\pm12.4)$ , monocytes  $(5.2\pm4)$ , eosinophils  $(7.5\pm2.4)$  and heterophils  $(26\pm11.5)$ . The overall prevalence and mortality rate was 15.34% and 10.34%. The proportional prevalence rate were 18.84%, 14.81%, 12.07%, 10.00% and mortality rate were 13.04%, 11.11%, 8.62% and 4.00%, respectively in age group of 0-4 weeks, 5-6 weeks, 7-8 weeks and above 8 weeks. The prevalence and mortality rate was highest in 0-4 week's age group (18.84% & 13.04%)) and lowest in above 8 weeks age group (10.0% and 4.0%). The recorded clinical signs were depression, ruffled feather, bloody diarrhea, anemia, drooping wings, pale comb and wattle. At necropsy, enlargement and bellowing shape of caecum with pin point hemorrhage on intestinal mucosa and fresh or clotted blood were found in the intestinal lumen. Histopathologically, the mucous membrane was severely damaged, mucosal discontinuity, distortion of architecture and desquamation of lining epithelium. Infiltration of inflammatory cell in musculature and mucosal villi was destroyed. The bio-safety measures, farmer's 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.

Keywords: Sonali chicken, Coccidiosis, Histopathology, Hematology.

### Introduction

Bangladesh is a developing country where poultry industry plays an important role in rural economy (Nath et al., 2014). Poultry sector employs about 5 million people & has experienced a long-term growth rate of about 4.50%, which is highest in the economy (BLRI report, 2019). Poultry rearing is an integral part of many farming systems and increasing day by day in Bangladesh. In Bangladesh, poultry is used for meat and egg production. It provides one of the main sources of income for the farmers of Bangladesh. Poultry rearing is considered unrivaled to the other agricultural sector in Bangladesh because of the quick economic return in a relatively short period of time. The duration of poultry rearing is very short and within 36-75 days it is ready for marketing and suitable for human consumption (Kamal et al. 2015). At present chicken comes up with 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 SK 2007). It has been estimated that in 1961 there were almost 4 billion domestic fowl whereas in 2019 there were almost 26 billion, a more that 6-fold growth in 60 years (Wilson RT, 2021).

There several constraints of poultry are production 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. Intestinal coccidiosis, caused by several species of Eimeria, is an economically important disease of poultry (Zhang and Zeng, 2005). Temperature and moisture are important factors in the epizootiology of coccidiosis and faulty waterers have been identified as one source of excess moisture. A moist litter with high water content and a heat of 25-30 °C enhances oocyst sporulation (Davies SFM and Joyner LP 1955). The hot and humid environment of poultry houses in Bangladesh provides an ideal circumstance for the sporulation of coccidia oocyst. Together with the high reproductive potential of the Eimerian

parasites, they can produce large number oocysts in litter in a relatively short period of time. A preliminary report on the occurrence of *Eimeria tenella*, *Eimeria necatrix* and *Eimeria maxima* as determined by the fecal examination of chicks by Mondal and Qadir (1978). Birds of all ages are prone to coccidiosis, but most birds become infected in the first few weeks of life (Chookyinox *et al.* 2009). Mortality is usually higher in young chicks because most *Eimeria spp* parasitize birds aged 3-18 weeks (Dakpogan *et al.* 2013)

Blood plays an important role in the transportation of nutrients, metabolic waste products and gases around the body; blood represents a means of assessing clinical and nutritional health status of animals (Olorode and Longe 2000). The haematological profiles are most commonly used in nutritional studies for various birds (Pavlak et. al. 2005). In addition, blood parameters help diagnoses of specific poultry pathologies and might serve as basic knowledge for studies in immunology and comparative avian pathology (Bonadiman et. al. 2009). Haematological profiles of chickens are correlated with a number of factors such as gender, nutrition, rearing temperature, stocking density and stress conditions (Yanagita et al. 2011). Other studies revealed that significant reduction in red and white blood corpuscles indicates haemolytic anaemia and exposes the birds to high risk of infection (Showkat et. al. 2021). However, the histopathological and hematological study of coccidiosis has not yet been investigated properly in sonali chicken at Dinajpur area. The present study was undertaken to create baseline data on haematological and pathological profiles of chicken affected with avian coccidiosis.

## **Materials and Methods**

### **Research area and period:**

The research work was conducted from January -June 2023, at different upazila of Dinajpur district and research area was situated in between latitude 24°34' and 24°97' N; longitude 88°21' and 88°89' E. (http://en.wekipedia.org). The laboratory work was carried out in department of pathology and parasitology at Hajee Mohammad Danesh Science and Technology University, Bangladesh.

### **Experimental sample (bird):**

Sonali chickens of different commercial poultry farms of research area were the sample of this experiment. Representative samples of diseased and dead birds (blood, feces, intestinal part like dudenum, jejunum, caecum) were collected randomly. A total of 15 farms (Sonali) were visited, among which 300 diseased and dead birds were examined but 45 birds were found to be positive for coccidiosis. The number of birds in the farms was variable ranging from 200 to 3000 and 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. Blood sample from infected birds was collected and some infected birds were taken to the pathology laboratory.



### 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. Hematological examination of affected birds.

### **Clinical Examination of sick birds:**

The general health condition and age of the chicken were recorded. The chicken was 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.

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#### Parasitological examination of faeces:

Fecal samples were collected directly from cloaca with spatula or freshly fallen feces from the affected flocks and also collected during the postmortem examination of the birds. The faecal samples were examined by direct smear technique and floatation technique as described by Soulsby (1982). The *Eimeria* oocyst was indentified on the basis of their structural characteristics as described by Springer (1997) and Soulsby (1982).

### Hematological examination:

Blood from ten affected birds were collected and test was performed at Check up diagnostic center in Dinajpur and observed some parameters such as red blood cell (RBC), packed cell volume (PCV), hemoglobin, eosinophil, monocytes, lymphocytes etc.

### **Experimental Layout:**



Figure 2. Schematic illustration of the experimental layout.

### Necropsy findings of suspected birds:

The necropsy was done on the suspected dead and diseased birds collected from different farms. 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.

### 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.

### **Statistical Methods:**

The data were recorded and analyzed statistically by using statistical software 'SPSS' (version 20). The results were expressed in percentage with P– value and significance was determined when P<0.05. The mean intensity was calculated and analyzed by F-variance test.

Prevalence (%) =

### Result

In the present study, 15 farms (Sonali chicken) of research area were visited; 300 diseased and dead birds were examined through clinical and pathological examination among which 45 birds were found to be positive for coccidiosis. 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 area. Blood of affected birds was collected and tested at Checkup diagnostic center. The results of different clinical, pathological and hematological examination are as follows.

# Clinical examination of suspected birds and examination of faeces:

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 Eimeria. sp. During clinical examination, clinical signs were depression and ruffled feather along with paler comb and wattle, attachment of feces around the vent and blood mixed feces. Sometimes drooping wings, less egg production, weight loss were also found during examination. field Bloodv diarrhea was considered to be a most important clinical sign. Faecal sample were examined under microscope following standard procedure and oocyst of Eimeria sp. was identified by their typical morphological feature (Figure 3).



**Figure 3. Clinical examination of suspected birds and examination of faeces;** (A) Depression and ruffled feather, (B) Bloody mixed feees, (C) Oocyst of Eimeria sp. (4x)

## Prevalence of Coccidiosis in Sonali chicken at different Upazila:

Prevalence of coccidiosis in Sonali chicken at different Upazila is presented in table 1. The highest prevalence was observed at Sadar upazila 17.46% and lowest 12.73% at Parbotipur upazila. Proportional prevalence rate of coccidiosis in different age group is shown in table 2 where 0-4 weeks shows highest prevalence (18.84%) and lowest (10.0%) at >8 weeks of age. Proportional mortality rate of coccidiosis in different age group is shown in table 3 where 0-4 weeks of birds show highest mortality rate (11.23%) and lowest (1.88%) at >8 weeks of age. Graphical presentation of prevalence at different upazila in Dinajpur district and mortality rate of avian coccidiosis in different age group is shown in (Figure 4).

### Table 1. Prevalence of Coccidiosis in Sonali chicken at different Upazila of Dinajpur district.

Name of Upazilla	No. of birds examined	No. of infected birds	Percentage (%)
Dinajpur Sadar	63	11	17.46
Chirirbandar	65	10	15.38
Parbotipur	55	7	12.73
Fulbari	62	9	14.52
Birampur	55	9	16.36
Total	300	46	15.34

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Age of birds (weeks)	No. of birds examined	No. of infected birds	Percentage (%)
$0 - 4^{\text{th}}$	138	26	18.84
$5^{\text{th}} - 6^{\text{th}}$	54	8	14.81
$7^{ m th}-8^{ m th}$	58	7	12.07
$>8^{th}$	50	5	10.00
Total	300	46	15.34

### Table 2. Prevalence of coccidiosis in different age group

### Table 3. Mortality rate of coccidiosis in different age group

Age of Birds (weeks)	No. of birds examined	No. of dead birds	Percentage (%)	<b>P-value</b>
$0-4^{\text{th}}$	138	18	13.04	
$5^{\text{th}}$ - $6^{\text{th}}$	54	6	11.11	0.018*
$7^{ m th}$ - $8^{ m th}$	58	5	8.62	
$>8^{\text{th}}$	50	2	4.00	
Total	300	31	10.34	

### Hematological findings:

Blood sample were collected from clinically coccidiosis infected chicken and subjected to hematological examination. The changes in hematological parameters affected birds were studied and recorded; the obtained data were comparing to the standard value indicated by Irizaary Rovira (2004) and Wakenell (2010). Coccidiosis caused by *Eimeria spp.* instigated a declining (mean  $\pm$  SD) in RBC (1.9 $\pm$  0.6) and PCV (24.3 $\pm$  5.1). The differential WBC (leukocyte) calculation showed an increase in lymphocytes (68.3 $\pm$ 15), monocytes (5.2 $\pm$ 4), eosinophils (8.1 $\pm$ 7) and heterophils (24.2  $\pm$ 13) (Table 4).



### Figure 4. Mortality rate of Coccidiosis in different age group

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Parameters	Infected chicken (Mean $\pm$ SD)	Normal Value of Healthy chicken
RBC(x106/mm3)	$1.9 \pm 0.6$	2.3-2.55 (Wakenell 2010)
PCV (%)	$24.3 \pm 5.1$	25-40 (Irizaary-Rovira 2004)
HB g/dl	9.0±2.1	8-14 (Wakenell 2010)
MCV (fL).	145.3±20.4	100-200 (Wakenell 2010)
MCH (pg)	55.1±12.1	30-45 (Wakenell 2010)
MCHC (%)	40.2±5.8	30-35 (Wakenell 2010)
Lymphocytes (%)	68.3±15	20-40 (MVM 2011)
Monocytes (%)	5.2±4	2-4 (MVM 2011)
Eosinophils (%)	8.1±7	1-6 (MVM 2011)
Heterophils (%)	24.2 ±13	30-70 (MVM 2011)

### Table 4. Blood cellular parameters in *Eimeria* infected sonali chicken (n = 10)

RBC = Red blood cells, PCV = Packed cell volume, HG = hemoglobin, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, Heterophils =band, MVM= Merck Veterinary Manual.

### **Necropsy findings:**

The suspected coccidiosis affected 33 dead and sick birds were subjected to postmortem examination and gross lesion of the various organs were studied and recorded. Necropsy findings in different intestinal regions of chicken were detected by postmortem examination. The identified findings were enlargement and bellowing shape caeca with profuse clotted blood Reddish brown and blood clotted intestinal contents were found in the lumen of caeca Pin point hemorrhage on intestinal mucosa (Figure 5).





**Figure 5. Post-mortem examination and gross lesion;** (A) Dehydrated carcass, (B) Examination of viscera, (C) Normal caeca, (D) Enlarged, discolored and bellowing shape caeca, (E) Pin Point Haemorrhage of Intestinal Mucosa, (D) Blood clotted intestinal contents in the caecal lumen.

#### **Histopathological study:**

For histopathological study, intestine and caeca were collected from coccidiosis infected chicken during post-mortem examination and subjected to histopathological examination. After examination the recorded histopathological changes was destruction of mucous membrane, discontinuation of intestinal mucosal layer, desquamation of lining epithelia and distortion of normal architecture; hemorrhage and reactive cell infiltration in epithelial layer; necrosis and hemorrhage around the invading gland and infiltration of inflammatory cells in the musculature.; degeneration of epithelial cells, glands and intestinal villi (Fig. 3).



Fig 3. Histopathological changes in caecum and intestine; (A) Hemorrhage in lining epithelia, (B) Destruction of normal architecture of caecal musculature (C) Reactive cell infiltration, (D) Degeneration of epithelial cells and glands.

## Discussion

Coccidiosis is the most prevalent intestinal parasitic disease of poultry throughout world. In chicken coccidiosis mixed infection with different *Eimeria* sp. is common but single species infection is rare. This study was carried out to investigate the prevalence, pathology and hematological parameters of coccidiosis afected birds at commercial farms of Dinajpur area on the basis of history, clinical sign, hematological test, gross and microscopic lesions.

Clinical manifestation of naturally infected chickens with coccidiosis was studied. The common clinical manifestations was bloody diarrhoea, anaemic carcass, attachment of faeces around the vent, blood mixed feces, depression and ruffled feather with paler comb and wattle (Figure 2). These findings are also supported by Taylor et al. (2007) and Williams (2006) 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 in this study. Similar findings were reported by Waxier (1998); but there were a small variation due to management of birds, location and others factor like ventilation, feeding, watering, time of vaccination etc.

There was a relationship found among the prevalence of coccidiosis at different upazila of Dinajpur district. The prevalence was maximum in Dinajpur SadarUpazila (17.46%) and minimum prevalence was found (12.73%) in Parbotipur and the overall prevalence was (15.34%). This observation more or less similar to those authors report where the prevalence of coccidiosis have been reported from Bangladesh by Talha et al., (2001) and Giasuddin, et al (2003) who reported (9.40%. 5.51%, 9.17% respectively). However, Belal (2018) repoted higher prevalence in Sirajgonj district of Bangladesh (35.5%). This relatively higher prevalence of coccidiosis could be described to the confinement and deep litterbased rearing system compared to caged birds.

The proportional prevalence of coccidiosis in different age group was 18.84%, 14.81%, 12.07%, and 10.00% and mortality rate was 13.04%, 11.11%, 8.62% and 4.00% in 0-4 weeks, 5-6 weeks, 7-8 weeks and above 8 weeks respectively which is similar to the observation of Kogut et al. (2005). Chapman et al. (2005) reported that, young birds are more susceptible and more rapidly display signs of disease, whereas older birds are relatively resistant. Chicks are not fully immunized and can experience great mortality in coccidiosis outbreak. The result is 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.

This study revealed that, coccidiosis causes a declining (mean  $\pm$  SD) in RBC (1.9 $\pm$  0.6) and PCV ( $24.3 \pm 5.1$ ). The differential leukocyte count (Table 1) showed an increase in lymphocytes  $(68.3\pm15)$ , monocytes  $(5.2\pm4)$ , eosinophils  $(8.1\pm7)$  and heterophils (24.2 ±13). Concerning the differential leukocyte count on broilers infected by E. tenella and E. brunetti, increased numbers of lymphocytes, monocytes, eosinophils and heterophils were obtained when compared with the reference value indicated by Merck Veterinary Manual (2011). The result is similar with those obtained by Fukata et al. (1997), who reported lower counts of RBC and PCV in chickens infected with E. tenella and E acervulina when they were compared to the uninfected controls. Ogbe et al. (2010) also reported a slight drop in the PCV, Hb and RBC counts in E. tenella infected broilers. Moreover, Razzaq et al. (2003) demonstrated the lowest Hb and total erythrocyte count (TEC) in quail chicks experimentally infected by E. tenella. Anemia, characterized by decreased PCV, RBCs, and/or Hb, is the most common erythrocyte abnormality in birds. Birds with a PCV less than 35% are generally considered anemic. The reduction in the RBC is due to the loss of blood into the gastrointestinal tract (external blood loss) and

infectious disease (Irizaary-Rovira, 2004). The results is similar to those report of Rose et al. (1979) who indicated that the peripheral blood leukocytes (PBL) response to infection with E. maxima and E. acervulina in chicken shows the increment in the number of PBL. Similar findings were also mentioned by Ricklefs and Sheldon (2007), who found the high counts of lymphocytes, heterophils and eosinophils in parasitic (malaria and haemosporidin) infected birds. In primary infections, the number of blood leukocytes increased biphasically and changes were found in the count of polymorphonuclear cells, lymphocytes and large mononuclear cells.

A total number of 33 dead and sick birds suspected to be infected with coccidiosis were subjected to postmortem examination and gross lesion of the various organs of infected chickens were studied. At necropsy, the major pathological lesions were found in intestinal caeca which was enlargement and ballowing shape of caecum with pin point hemorrhage in caecal wall and reddish brown or blood clotted intestinal contents in the lumen of caeca. These findings were in-agreement with the earlier report of Bertke (1989), Reid (2002) and Talukdar at el. (2017) who records the similar post-mortem changes in coccidiosis affected chicken. Present study also record the pin point hemorrhage on intestinal mucosa, hemorrhagic enteritis, mucoid to blood-tinged exudates and profuse mucosal bleeding in the caeca. This observation is similar to those report of Jagadeesh et al. (1976), Levine, (1983), Arakawa et al. (1981) and Paul (2009); but some differences were found in this study during postmortem examination of birds like discoloration and degeneration of internal organ. It may be due to time of death, other infectious or noninfectious diseases, delayed necropsy, environmental factors etc.

The recorded histo-pathological change of this study was severely damage mucous membrane, brake down in continuation of intestinal mucosal layer, distortion of architecture and desquamation of lining epithelia, necrosis and hemorrhage around the invading gland; degeneration of epithelial cells, glands, intestinal villi and infiltration of inflammatory cells in the musculature. This observation is similar to those findings of Jagadeesh *et al.* (1976), Noyilla *et al.* (2007) who reported that, the lining epithelia and villi of the mucosa were destroyed and disorganized; there was no continuation in the lining epithelium and reactive cell infiltration in mucosal layer. There was some variation with the findings of earlier report. It may be found due to strain, coloring agent, formalin, and other agent, lab environment etc.

## **Summary and Conclusion**

The present study was conducted to explore prevalence and haemato-pathological investigation of avian coccidiosis based on clinical. parasitological, necropsy, histopathological and hematological feature. Total 15 farms were visited among which 300 diseased birds were examined out of them 46 birds were found to be positive for coccidiosis. The recorded overall prevalence of coccidiosis in sonali chicken was 15.34% where as higher prevalence (17.46%) was recorded in sadar upzilla of Dinajpur district. the Among different age group, highest prevalence (18.84%) and mortality (13.04%) was recorded in 0-4 weeks of age. The clinical signs were recorded as bloody diarrhea, ruffled feather, depression, drooping wings, anemia, reduction of feed and water intake, pale or anemic carcass. 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. The present study demonstrated high susceptibility of Sonali chicken to coccidian (Eimeria sp.) infection. The severity of disease based on hematology to Eimeria infection was very high. It can be concluded that coccidiosis has a destructive effect on sonali chickens that is represented by a high reduction in RBC and PCV, increment in differential leukocyte counts and excessive tissue damage. The reported data make a contribution to the understanding of pathogenic mechanisms for coccidiosis in chicken. Coccidiosis can't be

accurately diagnosed based on clinical signs and lesions. Therefore, post-mortem laboratory support is essential for confirmatory diagnosis and antibiotics sensitivity tests. So, a field diagnostic laboratory for poultry disease should be developed and introduced at each Government Veterinary Hospital in Bangladesh. Due to the potential for a disastrous outbreak and the resulting financial loss, all young poultry should be vaccinated or given continuous medication with low levels of anticoccidial drugs. Finally, microbiological and molecular diagnoses are suggested for detailed studies of this disease and its pathogens and to develop sustainable and inexpensive prevention and control methods.

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