Hematological, Biochemical and Histopathological Changes Caused by Coccidiosis in Chickens

Meskerem Adamu1,2, Chaiwat Boonkaewwan1,*, Nirat Gongruttananun1 and Montakan Vongpakorn3

ABSTRACT

Hematological, biochemical and histopathological alterations caused by coccidiosis in broiler chickens from an outbreak of bloody coccidiosis in a flock on a small-scale broiler farm, Debre Zeit, Ethiopia, were evaluated. *Eimeria tenella* and *E. brunetti* were identified. Anemia caused by these species was characterized by a decreased number of red blood cells (RBC) and decreased packed cell volume (PCV). Differential leukocyte counts revealed monocytosis, lymphocytosis, heterophilia and eosinophilia. Serum biochemical analysis showed decreases in alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT) and aspartate aminotransferase/glutamic oxalacetic transaminases (AST/GOT), and a marked increase in alkaline phosphatase (ALP) activities. Histopathological examinations of the affected caeca also demonstrated excessive tissue damage, hemorrhage, the presence of clusters of large schizonts and merozoites in the tissue, and coccidial oocysts in the lumen. The study demonstrated changes in the hematology, histopathology and blood chemistry of broilers caused by *E. tenella* and *E. brunetti*.

Keywords: coccidiosis, histopathology, hematology, biochemistry, broiler

INTRODUCTION

Confinement rearing and high density flocks of commercial poultry have increased the exposure to diseases such as coccidiosis. The protozoan parasite of the genus *Eimeria* multiplies in the intestinal tract and causes tissue damage, resulting in the interruption of feeding, digestive processes, nutrient absorption, dehydration, blood loss, loss of skin pigmentation and increased susceptibility to other disease pathogens (McDougald, 2008). The short, direct life cycle and high reproductive potential of coccidians in poultry often leads to severe outbreaks of disease in small backyard flocks or in the modern poultry house (McDougald and Fitz-Coy, 2008). Coccidiosis is a disease of major economic importance in the poultry industry (Williams, 2005). Infection with coccidia parasites costs the poultry industry in the USA more than USD 1.5 billion in annual losses (Yun et al., 2000). It is a widespread disease in growing chickens around the world that can seriously restrict the development of poultry production. Coccidians consist of a wide variety of single cell parasitic animals in the sub-kingdom Protozoa, phylum...
Apicomplexa. Nine different species are known; of these, seven *Eimeria* occur in chicken—namely, *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella* (Conway and Mckenzie, 2007). *E. tenella* is highly pathogenic and causes bloody caecal coccidiosis (Kaufmann, 1996).

The diagnosis of coccidiosis is based on clinical signs, coprology and pathomorphological and pathohistological analysis (Long and Joyner, 1984; Conway and Mckenzie, 2007). In recent years, various biochemical and molecular methods have also been used (Morris and Gasser, 2006). Although serology is the predominant method of disease monitoring in commercial poultry, examination of blood smears, bone marrow and clinical chemistry values is rarely done (Wakenell, 2010). Current understanding of avian clinical biochemistry is in the early stages compared with knowledge of biochemical analysis in mammals. Serum/plasma enzyme activity is primarily used to determine if a pathological process, cellular injury and/or necrosis is presented. In addition, it also helps to localize the disease process to a particular cell type (Irizaary-Rovira, 2004). The specific diagnosis of infection plays a key role in the prevention, surveillance and control of coccidiosis. The purpose of this study was to evaluate hematological, biochemical and histopathological changes in broiler chickens naturally infected with bloody coccidiosis.

**MATERIALS AND METHODS**

**Study animal and sample collection**

An outbreak of intestinal disease occurred in a flock aged 5 wk and with a total of 1,000 chicks per flock kept at a small-scale broiler farm, Debre Zeit, Ethiopia. Chickens were vaccinated against Newcastle disease and infectious bursal diseases; however, no anticoccidial treatments were applied until the clinical signs appeared. Blood samples and intestinal and caecal tissues were collected from 10 prominently ill chickens.

**Hematological analysis**

Blood samples were collected from the brachial vein of 10 birds using a 3 mL sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the anticoagulant, ethylenediaminetetraacetic acid. The total red blood cell (TRBC) or erythrocyte counts were performed in a 1:200 dilution of blood in Hayem’s solution. The differential leukocyte counts were

**Identification of Eimeria species**

Necropsy was performed after blood collection. *Eimeria* species were identified by a combination of oocyst size, location in the gut, appearance of the lesions, and schizont size (Conway and Mckenzie, 2007; McDougald and Fitz-Coy, 2008). Mucosal scrapings and tissues were examined using a light microscope. *Eimeria* oocysts were isolated from caecal and lower intestinal mucosa using saturated sodium chloride floatation solution following the procedures mentioned by Permin and Hansen (1998).

**Histopathological examination**

Intestinal and caecal samples were diagnosed at the National Animal Health, Diagnostic and Investigation Center, Sebeta, Ethiopia. The tissue samples were fixed in 10% neutral formalin for histopathological examination. In brief, tissues were trimmed to 3 to 5 µm thickness and then processed in an automatic tissue processor in different chambers containing different alcohol concentrations (70, 80, 95 and 100%). The processed tissues were cleared in xylene and embedded in paraffin for preparation into fine blocks. Blocks were sectioned with a microtome to a size of 5 µm; afterward they were dewaxed and the tissues section was stained using haematoxylin and eosin (H and E) stain as described by Bancraft *et al.* (1990). The slides were mounted with distrene plasticizer xylene and allowed to dry before examination under a light microscope.
determined by preparation of blood smears stained with Wright’s stain. The Hb concentration was evaluated by matching acid hematin solution against a standard colored solution found in Sahl’s hemoglobinometer. Packed cell volume (PCV) was measured by a standard manual technique after centrifugation of a small amount of blood using microhematocrit capillary tubes. Red blood indices, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentrations (MCHC) were calculated from RBC, PCV and Hb, respectively (Coles, 1986; Irizaary-Rovira, 2004).

**Biochemical analysis**

Blood was collected without anticoagulant for serum biochemistry determination. Serum was separated after centrifugation at 3,000 rpm for 15 min and stored at -20 °C until used. Serum alanine aminotransferase/glutamic pyruvic transaminase (ALT/GPT), aspartate aminotransferase/glutamic oxalacetic transaminases (AST/GOT) and alkaline phosphatase (ALP) activities were measured according to the manufacturer’s instruction (FVM-AAU; Addis Ababa, Ethiopia).

**Data analysis**

The statistical analysis system (SAS, 2000) was used to determine the descriptive statistics of the mean, range and standard deviation of hematological and serum biochemical data. The mean data were analyzed by a comparison with the reference interval value.

**RESULTS**

*E. tenella* and *E. brunetti* were identified from caecal and lower intestinal mucosa, respectively, of broiler chickens suffering from bloody coccidiosis. Infected chickens showed typical signs of coccidiosis including bloody diarrhea and weight loss. Necropsy showed enlarged and distended caeca filled with blood and petechial hemorrhages in some parts of the lower intestine (Figure 1). *E. tenella* was identified easily by its predilection site (caeca), characterized lesions (bleeding), ovoid oocysts and clusters of large schizonts. The oocyst count per gram of faeces from scrapings of caecal mucosa was more than 100,000. *E. brunetti* was identified by its location, lesion found at the lower intestine,

![Figure 1](image_url) **Figure 1** Gross lesion of *E. tenella*-infected broiler chicken caeca. The unopened caecum is distended with blood (shown by arrow).
the presence of petechial hemorrhages and ovoid oocysts. However, no schizonts were identified and only a small number of oocysts were found (5,000).

Microscopic examinations of the affected caeca showed severe tissue damage and plenty of schizonts and oocysts (Figures 2A–2D).

Coccidiosis caused by *E. tenella* and *E. brunetti* induced a reduction (mean ± SD) in RBC (1.7 ± 0.42) and PCV (23.4 ± 4.2). The differential WBC (leukocyte) count (Table 1) demonstrated an increase in lymphocytes (64.6 ± 13), monocytes (4.6 ± 5), eosinophils (7.3 ± 5) and heterophils (23.3 ± 11). The data showed decreased levels

![Figure 2](image_url)

**Figure 2** Section of *E. tenella*-infected broiler chicken caeca, showing cluster of large schizonts (arrow) 40× (a), necrosis and disintegration of glandular epithelial cells (arrow) 10× (b), hemorrhage in the sub-mucosa (arrow) 10× (c), merozoites (line) and oocysts (arrow) in the mucosa and tissue 40× (d). Haematoxylin and eosin (H and E) stain used.
in the biochemical serum ALT (6.5 ± 2.2), AST (121.5 ± 35.2) and a marked increase in the enzyme ALP (1392.8 ± 23) activities in *E. tenella* and *E. brunetti* infected broilers (Table 2).

**DISCUSSION**

*E. tenella* and *E. brunetti* were previously reported from different places in Ethiopia by several investigators; for instance Lobago et al. (2005) identified *E. brunetti* in the Kombolcha Poultry Multiplication and Research Center (KPMRC) while Getachew (2004), Lobago et al. (2005) and Mersha et al. (2009) reported *E. tenella* in Tiyo Wereda, Arsi Administrative Zone of Oromia Regional State, KPMRC and in three commercial broiler farms of Debre Zeit, Central Ethiopia. *Eimeria* species identified in the present study were *E. tenella* and *E. brunetti*. Large numbers of *E. tenella* oocysts and clusters of large schizonts were detected; however, only small numbers of *E. brunetti* oocysts were found and no schizonts were detected. The severity of coccidial infection may vary with the isolate, number of oocysts ingested and the immune state of the bird. Some species are identified easily by the location and appearance of gross lesions in concert with the size of oocysts or schizonts (*E. acervulina, E. maxima, E. necatrix, and E. tenella*). The presence of clusters of large schizonts in the caecum is pathognomonic for *E. tenella*. *E. brunetti* oocysts are indistinguishable from those of *E. praecox, E. tenella* and *E. necatrix* based on size alone but the location in the lower gut and the appearance of the lesions could be used as reliable indicators. Light infections of *E. brunetti* are overlooked easily unless careful attention is paid to the lower small intestine. Histopathology of *E. brunetti* reveals shizonts on the fourth day of infection. (Kaufmann, 1996; McDougald and Fitz-Coy, 2008).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Blood cellular parameters in <em>E. tenella</em> and <em>E. brunetti</em> infected broilers (n = 10).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>RBC (1×10⁶ cells per microliter)</td>
<td>1.7 ± 0.42</td>
</tr>
<tr>
<td>HB (g.dL⁻¹)</td>
<td>8.7 ± 1.7</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>138.2 ± 18.4</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>52.0 ± 11.4</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>37.2 ± 4.71</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>64.6 ± 13</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>4.6 ± 3</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>7.3 ± 5</td>
</tr>
<tr>
<td>Heterophils (%)</td>
<td>23.3 ± 11</td>
</tr>
</tbody>
</table>

MVM = Merck Veterinary Manual (2011), 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.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Serum biochemical parameters in <em>E. tenella</em> and <em>E. brunetti</em> infected broilers (n = 10).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable (IU.L⁻¹)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>6.5 ± 2.2</td>
</tr>
<tr>
<td>Aspartate aminotransferase (AST)</td>
<td>121.5 ± 35.2</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>1392.8 ± 23</td>
</tr>
</tbody>
</table>

Gross and microscopic pathology were specifically used to demonstrate the severity of the disease in chickens naturally infected with *E. tenella* and *E. brunetti*. The presence of high numbers of oocysts, schizonts and severe tissue damage in the caeca indicated the severity of infection due to *E. tenella*. Histopathological examination of the affected caeca showed similar findings with those reported by McDougald and Fitz-Coy (2008), who described the most pathogenic stage caused by *E. tenella* as the second generation schizont, which caused excessive tissue damage, bleeding, disruption of the caecal glands and destruction of the mucosa and muscularis layer. Microgametocytes and macrogametocytes of schizonts are seen in the tissue on days 6 and 7 after infection and matured oocysts are released into the lumen in huge numbers. The present results were similar to those mentioned by Getachew (2004) and Mersha *et al.* (2009) who studied *E. tenella*-infected local and RIR chickens, and *E. tenella* - and *E. acervulina*-infected broiler chickens, respectively.

Comparative results of the obtained data and the standard value indicated by Irizaary-Rovira (2004) and Wakenell (2010) showed that coccidiosis caused by *E. tenella* and *E. brunetti* induced a higher reduction in TRBC and PCV (Table 1). These results are similar with those obtained by Fukata *et al.* (1997), who reported lower counts of TRBC 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).

Concerning the differential WBC (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 present results were similar to those reported by 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. In primary infections, the number of PBL increased biphasically and changes were found in the count of polymorphonuclear cells, lymphocytes and large mononuclear cells. 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.

The increase in the lymphocyte count may be attributed to the effect of the inflammation of the caeca and intestine. Chronic antigenic stimulation may result in a greatly expanded circulating lymphocyte pool because the primary functions of the lymphocytes are immunological response, humoral antibody formation and cell mediated immunity (Irizaary-Rovira, 2004). Antibody-mediated responses play a minor role in protection against coccidiosis. There is increasing evidence that cell-mediated immunity plays a major role in resistance to infection as T lymphocytes appear to respond to coccidial infection through both cytokine production and a direct cytotoxic attack on infected cells (Lillehoj and Trout, 1996; Yun *et al.*, 2000). *E. tenella* infection seems to be rapidly induced locally at the site of the parasite development in an increased proportion to the CD4+ cells on day 8 post infection and CD8+ cells on days 6 and 8 post infection in caecal intraepithelial lymphocytes of infected chicken (Bessay *et al.*, 1996).
Eosinophilia in birds rarely occurs but may be associated with parasitism (mites, intestinal parasites, parasites with tissue migration) according to Irizaary-Rovira (2004). Eosinophils are known to interact with homocytotropic antibodies (IgE and IgG), mast cells and basophils. The antibody and T lymphocytes provide specificity to the reaction and the IgE on mast cells attracts eosinophils to modulate the inflammatory reaction. The relative quantities of tissue IgE, extractable histamine, and eosinophil suggest that these components form a system which is most dominant on body surfaces, immunologically mediated, often parasite related and frequently associated with eosinophilia (Ted, 2007).

Heterophils also contain a variety of granules that contribute to the first line host defense against bacteria, fungi, protozoa and some viruses (Wakenell, 2010). Acute or chronic inflammatory disease is the predominant cause of monocytosis or heterophilia in pet birds (Irizaary-Rovira, 2004) because monocytes, macrophages and dendritic cells are important hematopoietic cells that play critical roles in defense and in maintaining homeostasis. Wakenell (2010) noted that the majority of inflammatory tissue macrophages arise from monocytes recruited from blood and that regardless of location, tissue macrophages have similar functions which include surveillance, removal of dead cells and cellular debris, defense against pathogens, promotion of wound healing and tissue remodeling and repair.

The present study also showed decreases in ALT and AST and a marked increase in enzyme ALP activities in *E. tenella* and *E. brunetti* infected broilers (Table 2). These results are similar to the result of Mondal *et al.* (2011) who reported that ALT decreased in broiler chickens infected with a field isolate of *E. tenella*. On the other hand, the present findings were different from the previous studies indicated by Biu *et al.* (2006), who reported that the ALT level was increased while ALP activity was decreased in mixed coccidian-infected chickens. Mondal *et al.* (2011) demonstrated that plasma AST activity was increased in infected broiler chickens with a lower dose of *E. tenella*. A decrease in plasma enzyme levels is much less frequently used for clinical interpretation. However, there are a few specific cases where low plasma enzyme levels will indicate that the relevant organ is hyperplastic, atrophied or destroyed (Kerr, 2002). ALT and AST are the enzymes found in erythrocytes; therefore, the decrease in the activities of serum ALT and AST reported in the present study may be associated with the high reduction of erythrocytes because of the loss of blood into the gastrointestinal tract.

Unfortunately, ALP, a serum enzyme associated with bile ductular epithelium and cholestasis in mammals, is not specifically associated with biliary epithelium in birds (Irizaary-Rovira, 2004). Alkaline phosphatase is found particularly in bone (osteoplates), liver and the intestinal wall, with high levels being found in young animals with high osteoblastic activity (Kerr, 2002). Markedly increased plasma activities are particularly associated with parasite-induced damage to the intestinal wall in horses, though an elevation of this enzyme activity is normal in young birds (Kerr, 2002). The noticeably increased serum activities of ALP found in the present study might be associated with the metabolic alteration and damage of the bone marrow as compensation for the blood losses; the bone marrow might be forced to produce excessive blood cellular components.

**CONCLUSION**

The present study demonstrated the high susceptibility of broilers to coccidian infection caused by *E. tenella* and *E. brunetti*. The severity of the disease based on hematology, blood chemical responses and histopathology to *E. tenella* and *E. brunetti* infection was very high. It can be concluded that coccidiosis caused by these species has a destructive effect on broiler chickens that is represented by a high reduction in RBC and
PCV, increment in differential leukocyte counts, reduction in serum ALT/GPT, AST/GOT, markedly increased ALP activities and excessive tissue damage. The reported data make a contribution to the understanding of the pathogenic mechanisms of coccidiosis in chicken. 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.

**LITERATURE CITED**


Health Prod. 41: 1309–1317.


