

Hemato-Biochemical and Histopathological Changes Caused by Coccidiosis in broilers.

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Abstract:

The aim of present work is to determine the Hematological, biochemical and histopathological alterations caused by coccidiosis in broiler chickens from an outbreak of bloody coccidiosis in a flock. The study was conducted on Al bahrani field for commercial broiler in Al Najaf province-Iraq. Blood samples were collected from jugular vein into EDTA tubes for hematological value and plain tube for biochemical value during October 2017 . Result showed that the Anemia caused by the coccidian was characterized by a decreased number of red blood cells (RBC) and decreased packed cell volume (PCV). Differential leukocyte counts revealed to increase in monocytes, lymphocytes, heterophil and eosinophil. Also, Serum biochemical analysis showed decreases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and a marked increase in alkaline phosphatase (ALP) activities. On the other hand, Histopathological examinations of the affected caeca also demonstrated excessive tissue damage, hemorrhage, the crypt cells were highly invaded with the developmental stages of *E. tenella* schizonts and gametocytes that their morphology is practically missing. In conclusions the present study revealed changes in the hematology, blood chemistry and histopathology of broilers caused by *E. tenella* and *E. brunetti*.

Keywords: coccidiosis, histopathology, hematology, broiler

التغيرات المرضية الدموية والكيميائية والنسجية الناجمة عن الإصابة بداء الكوكسيديا في الدجاج اللحم

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الخلاصة:

الهدف من الدراسة الحالية هو تحديد التغيرات الدموية والكيميائية والنسجية المرضية الناجمة عن الإصابة بالكوكسيديا في دجاج اللحم، وأجريت الدراسة على حقل في محافظة النجف - العراق. تم جمع عينات الدم من الوريد الودجي إلى أنابيب EDTA لتقييم مكونات الدم و الأنبوب العادي لتقييم البيوكيميائية للمصل خلال شهر أكتوبر 2017. أظهرت النتائج أن فقر الدم الناجم عن الكوتسيديان يتميز بانخفاض عدد خلايا الدم الحمراء وانخفاض حجم الخلايا المضغوط (PCV). وكشفت العد الفريقي لكريات الدم البيضاء الى زيادة في الخلايا وحيدة النواة، الخلايا الليمفاوية، الهيتيروفيل والحمضيات. كما أظهر التحليل البيوكيميائي لمصل الدم انخفاضا في ناقلات الأمين ألانين (ALT) و أسبارتات أمينوترانزفيراس (AST)، وزيادة ملحوظة في أنشطة الفوسفاتيز القلوية (ALP). من ناحية أخرى، أظهرت الفحوص النسجية للاعورين المصابة أيضا تضرر كبير لأنسجتها، والنزف، وجود مجاميع كبيرة من الشيزوننتس و الميروزويتات في بطانة الاعورين. استنتجنا من الدراسة الحالية وجود تغيرات في خلايا الدم ومكونات المصل ونسجيا بسبب الإصابة الايميريا *E. tenella* and *E. brunetti* في حقل لتربية الدجاج اللحم

Introduction :

Coccidiosis, is a result parasites related to the genus *Eimeria*, it is one of the excessive imperative poultry diseases worldwide. High morbidity and mortality, decline in feed efficiency, body weight gain, entirely contribute to the economic importance (1). Coccidiosis is a malady of major monetary significance in the poultry industry (2). Infection with coccidia parasites costs the poultry industry in the USA more than USD 1.5 billion in yearly misfortunes (3). It is an across the board illness in developing chickens around the globe that can genuinely limit the improvement of poultry creation. Coccidians comprise of a wide assortment of single cell parasitic creatures in the sub-kingdom Protozoa, phylum Apicomplexa. Nine unique species are known; of these, seven *Eimeria* happen in chicken to be specific, *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox* and *E. tenella* (4). *E. tenella* is very pathogenic and causes bleeding in cecum (5). The finding of coccidiosis depends on clinical, coprological and pathomorphological signs and pathohistological investigation (4). As of late, different biochemical and atomic techniques have additionally been utilized (7). Despite the fact that serology is the prevalent strategy for infection observing in commercial poultry, examination of blood smears, bone marrow and clinical science esteems is infrequently done (8). Current comprehension of avian clinical organic chemistry is in the beginning periods contrasted and information of biochemical investigation in vertebrates.

Materials and Methods:**Study animal and sample collection**

An outbreak of intestinal disease occurred in a flock aged 7 week and with a total of 1000 chicks. Chickens were vaccinated against Newcastle disease and infectious bursal diseases; however, no anticoccidial

treatments were applied until the clinical signs appeared. Blood samples were collected from jugular vein for hematological and biochemical investigation. Also, intestinal and caecal tissues were collected from 50 prominently ill chickens.

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 (28). 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 (29).

Histopathological examination

Intestinal and caecal samples were diagnosed at laboratory of college veterinary medicine in university of kufa. 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 (30). The slides were mounted with distrene plasticizer xylene and allowed to dry before examination under a light microscope.

Hematological analysis

Blood samples were collected from the jugular vein of 50 birds using a 5 mL sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the

anticoagulant. 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 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 rohematocrit capillary tubes. Red blood indices, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentrations (MCHC) were calculated from RBC, PCV and Hb, respectively (15).

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 (ALT), aspartate aminotransferase/ (AST/) and alkaline phosphatase (ALP) activities were measured according to the manufacturer's instruction

Data analysis

The data were analyzed with SPSS 16.0 for windows by using a one-way analysis of variance (10). Differences between means were determined using Tukeys test at $P < 0.05$ level.

Results

E. tenella and *E. brunetti* were distinguished from lower intestinal mucosa and cecum, respectively, of broilers suffering from bloody coccidiosis. Infected broilers showed typical signs of coccidiosis including blood stained diarrhea and weight reduction. Post mortem indicated amplified and widened caeca loaded with blood and petechial hemorrhages in a few sections of the lower digestive tract (Figure 1). *E. tenella* was recognized effectively by its inclination site (caeca), The lesions include hemorrhages, ovoid oocysts and clusters of large schizonts. The oocyst count pergram of faeces from scrapings mucosa of caeca was more than 90,000. *E. brunetti* was recognized by its area, lesion found at the lower digestive system, the manifestation of petechial hemorrhages and ovoid oocysts.



Figure 1 Post Mortem of *E. tenella*-infected broiler chicken caeca. The opened caecum is distended

With bloody caecal content becomes thicker, mixed with fibrinous exudate and acquires a cheese like appearance.

Furthermore, no schizonts recognized and just few oocysts were found (3,500). Microscopic examinations of the influenced caeca demonstrated extreme tissue destruction and abundance of schizonts and oocysts (Figures 2).

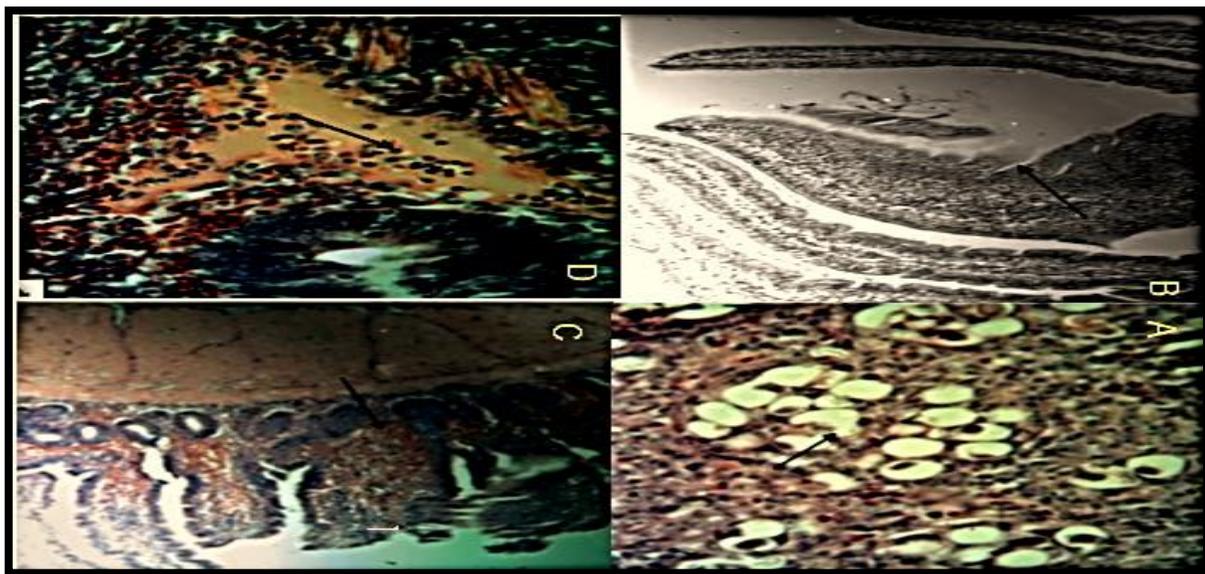


Figure 2 Section of *E. tenella*-infected broiler chicken caeca, showing cluster of large schizonts (arrow) 40 \times (a), necrosis and disintegration of glandular epithelial cells (arrow) 10 \times (b), hemorrhage in the sub-mucosa (arrow) 10 \times (c), merozoites (line) and oocysts (arrow) in the mucosa and tissue 40 \times (d). Haematoxylin and eosin (H and E) stain used.

Coccidiosis caused by *E. tenella* and *E. brunetti* instigated a declining (mean \pm SD) in RBC (1.9 ± 0.6) and PCV (24.3 ± 5.1). The differential WBC (leukocyte) calculation (Table 1) showed an increase in lymphocytes (68.3 ± 15), monocytes (5.2 ± 4), eosinophils (8.1 ± 7) and heterophils (24.2 ± 13).

Table 1 Blood cellular parameters in *E. tenella* and *E. brunetti* infected broilers (n = 10).

parameters	Mean \pm SD
RBC(x106/mm3)	1.9 \pm 0.6
PCV (%)	24.3 \pm 5.1
HB g/dl	9 \pm 2.1
MCV (fL).	145.3 \pm 20.4
MCH (pg)	55.1 \pm 12.1
MCHC (%)	40.2 \pm 5.8
Lymphocytes (%)	68.3 \pm 15
Monocytes (%)	5.2 \pm 4
Eosinophils (%)	8.1 \pm 7
Heterophils (%)	24.2 \pm 13

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

Table 2 showed that ALT, AST were decreased levels in the biochemical serum ALT

And a marked increase in the enzyme ALP activities in *E. tenella* and *E. brunetti* infected broilers.

Table 2: The enzyme activities in *E. tenella* and *E. brunetti* infected broilers

Parameter (U/L)	Mean \pm SD
Alanine aminotransferase (ALT)	8.5 \pm 1.025
Aspartate aminotransferase (AST)	180 \pm 20.16
Alkaline phosphatase (ALP)	1513 \pm 154

Values are mean \pm SD (n=10).

Discussion

E. tenella and *E. brunetti* were formerly described from changed residences by a few examiners; for example (11), (12), (13) detailed *Eimeria tenella*. *Eimeria* species distinguished in the present investigation were *E. tenella* and *E. brunetti*. Extensive quantities of *E. tenella* oocysts and groups of vast schizonts were distinguished; nonetheless, just little quantities of *E. brunetti* oocysts were found and no schizonts were distinguished. The seriousness of coccidial contamination may fluctuate with the separate, number of oocysts ingested and the resistant state of the bird. A few animal varieties are recognized effortlessly by the area and appearance of gross lesions working together with the span of oocysts or schizonts (*E. acervulina*, *E. maxima*, *E. necatrix*, and *E. tenella*). The nearness of bunches of substantial schizonts in the caecum is pathognomonic for *E. tenella*. *E. brunetti* oocysts are vague from those of *E. praecox*, *E. tenella* and *E. necatrix* in view of size alone yet the area in the lower gut and the presence of the sores could be utilized as dependable pointers. microscopic pathology of *E. brunetti* uncovers schizonts on the fourth day of disease. (14), (1). Gross and Histopathology were particularly used to exhibit the seriousness of the illness in chickens normally tainted with *E. tenella* and *E. brunetti*. The nearness of high quantities of oocysts, schizonts and extreme tissue harm in the caeca showed the seriousness of disease because of *E. tenella*. Histopathological examination of the influenced caeca indicated comparative discoveries with those announced by (1), who portrayed the most pathogenic stage caused by *E. tenella* as the second era schizont, which caused over the top tissue harm, bleeding, interruption of the caecal glands and devastation of the mucosa and muscularis layer. Microgametes and

macrogametes of schizonts are found in the tissue on days 6 and 7 after contamination and developed oocysts are discharged into the lumen in gigantic numbers. The present outcomes were like those specified by (11) and (13) who considered *E. tenella* tainted nearby, and *E. tenella*-and *E. acervulina* tainted broiler chickens, separately. Near aftereffects of the acquired information and the standard esteem demonstrated by (15), (8) demonstrated that coccidiosis caused by *E. tenella* and *E. brunetti* prompted a higher diminishment in TRBC and PCV (Table 1). These outcomes are comparable with those acquired by (16), who detailed lower include of TRBC and PCV in chickens contaminated with *E. tenella* and *E. acervulina* when they were contrasted with the uninfected controls. (17) additionally announced a slight drop in the PCV, Hb and RBC totals in *E. tenella* infected broilers. In addition, (18) showed the most minimal Hb and aggregate erythrocyte include (TEC) quail chicks experimentally infected by *E. tenella*. Blood deficiency, characterized by reduced PCV, RBCs, as well as Hb, is the most communal erythrocyte anomaly in fowls. Birds with a PCV less than 35% are commonly considered anemic. The depletion in the RBC is because of the loss of blood into the gastrointestinal tract and infectious disease (15). Concerning the differential WBC (leukocyte) count on broilers tainted by *E. tenella* and *E. brunetti*, bigger numbers of lymphocytes, monocytes, eosinophils and heterophils were obtained when contrasted and the reference rate showed by (19). The present outcomes were like those detailed by (20), who demonstrated that the peripheral blood leukocytes (PBL) reaction to infection with *E. maxima* and *E. acervulina* in chicken demonstrates the augmentation in the quantity of PBL. In imperative diseases, the quantity of PBL expanded biphasically and

changes were found in the count of polymorphonuclear cells, lymphocytes and substantial mononuclear cells. Comparable discoveries were likewise said by (21), who found the high totals of lymphocytes, heterophils and eosinophils in parasitic (malaria and haemosporidin) infested fowls. The expansion in the lymphocyte total may be ascribed to the impact of the irritation of the caeca and digestive tract. Chronic antigenic incitement may bring about an extraordinarily extended flowing lymphocyte pool on the grounds that the primary functions of the lymphocytes are immunological reaction, humoral antibody creation and cell mediated resistance (15). Antibody mediated responses reactions show a minor part in defense against coccidiosis. There is expanding evidence that cell-mediated immunity assumes a significant role in protection from infection as T lymphocytes seem to react to coccidial disease through both cytokine creation and a direct cytotoxic assault on tainted cells (22),(3). *E. tenella* disease is by all accounts quickly initiated locally at the site of the parasite improvement in an expanded extent to the CD4+ cells on day 8 post disease and CD8+ cells on days 6 and 8 post contamination in caecal intraepithelial lymphocytes of tainted chicken (23). Eosinophilia in fowls rarely happens but may be related with parasitism (flies, intestinal parasites, parasites with tissue relocation) as per (15). Eosinophils are acknowledged to intermingle with homocytotropic antibodies (IgE and IgG), mast cells and basophils. The antibody and T lymphocytes afford specificity to the response and the IgE on mast cells attracts eosinophils to adjust the inflammatory response. The relative amounts of tissue IgE, extractable histamine, and eosinophil recommend that these segments frame a framework which is most noticeable on body surfaces, immunologically interceded,

regularly parasite related and habitually connected with eosinophilia (24). Heterophils likewise contain an assortment of granules that add to the main line have protection against bacteria, fungi, protozoa and some viruses (8). Acute or chronic provocative condition is the prevalent reason for monocytosis or heterophilia in pet birds (15) in light of the fact that monocytes, macrophages and dendritic cells are imperative hematopoietic cells that assume basic parts in protection and in looking after homeostasis. (8) noticed that the greater part of incendiary tissue macrophages emerge from monocytes selected from blood and that paying little respect to area, tissue macrophages have comparative capacities which incorporate observation, expulsion of dead cells and cell flotsam and jetsam, barrier against pathogens, advancement of wound mending and tissue redesigning and repair.

Biochemical serum analysis of this present study showed decreases in ALT and AST and a obvious increase in enzyme ALP happenings in *E. tenella* and *E. brunetti* infested broilers (Table 2). These consequences are alike to the result of (25) who described that ALT decreased in broiler chickens infected with a field isolate of *E. tenella*. Conversely, the current results were altered from the prior revisions showed by (26), who reported that the ALT level was increased while ALP activity was decreased in mixed coccidian-infected broilers. (25) revealed that plasma AST activity was augmented in infected chickens with a lesser dosage of *E. tenella*. Nevertheless, there are a few specific circumstances where little plasma enzyme levels will indicate that the relevant organ is hyperplastic, atrophied or destroyed (27). ALT and AST are the enzymes create in erythrocytes; hence, the reduction in the activities of serum ALT and AST informed in the current study may be associated with

the high decrease of erythrocytes because of the loss of blood into the gastrointestinal passage. Alkaline phosphatase is established mainly in bone (osteoplasts), liver and the intestinal wall, with high levels present in new animals with high osteoblastic activity (27). The markedly increased serum activities of ALP initiate in the present study might be associated with the metabolic variation and destruction of the bone marrow as recompense for the blood losses; the bone marrow might be enforced to produce extreme blood cellular constituents.

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