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## Exploring the pathological hallmarks of naturally occurring colibacillosis in broiler chickens reared in North Kashmir

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### Abstract

The aim of the study was to examine the hematological, serological and Pathomorphological changes in the naturally occurring Colibacillosis in broiler chickens during the period of March to August, 2023 in commercial broiler farms at Ganderbal district of Kashmir. During this study, a total of 200 cases of Colibacillosis were identified at 10 different broiler farms and the dead birds were sent to the Division of Veterinary Pathology for a thorough postmortem investigation. Colibacillosis is a complex disease mainly occurring in the age group of 3–4 week age of age and is characterized by air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis or yolk sac infection. The pathological changes occurring in *Escherichia coli* infection were studied in natural cases of avian Colibacillosis. The samples were collected from different poultry farms of Ganderbal district of Kashmir. During postmortem examination, fibrin deposition on the liver and heart, as well as congestion in different organs were observed in the affected birds. The histopathological examination revealed necrosis and the depletion of lymphocytes in the spleen, interstitial pneumonia, fibrinous pericarditis, myocarditis, fibrinous perihepatitis, hepatitis and fatty degeneration in the hepatocytes. It is concluded that the pathogenic *E. coli* in poultry led to systemic lesions and immunosuppression in birds.

**Keywords:** *Escherichia coli*, chicken, pathological lesions, Colibacillosis

### Introduction

*Escherichia coli* are thought to be the primary or secondary pathogen responsible for avian Colibacillosis, which is one of the main causes of morbidity and mortality in poultry (Lutful Kabir, 2010) [17]. The gas exchange regions of the lung and the air sacs are thought to be sites of entrance *Escherichia coli* into the circulation because they lack resident macrophages, making them relatively susceptible to bacterial invasion and colonization (Mellata *et al.*, 2003) [19]. All age groups of poultry are susceptible to these diseases, but broiler chicks within the first four to six weeks of life are particularly vulnerable and seriously affected, with a high death rate (Leitner and Heller, 1992) [18]. Colibacillosis is a complex syndrome characterized by multiple organ lesions like air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis and yolk sac infection. The concomitant infection of *E. coli* and the turkey rhinotracheitis virus frequently causes swollen head syndrome in hens (Stehling *et al.*, 2003) [25]. Colibacillosis is considered one of the main causes of morbidity and mortality in poultry worldwide. There are significant financial costs associated with respiratory tract infections, particularly the colisepticemia which is often followed by septicemia (Barnes and Gross, 1997 and Ewers *et al.*, 2003) [4, 9]. Colisepticemia usually results in death, but certain birds can recover fully or only partially, resulting in meningitis, panophthalmitis, osteoarthritis, synovitis and coligranuloma characterized by multiple granulomas in the liver, caecum, duodenum, and mesentery (Barnes *et al.*, 2003) [2]. Keeping in view the paucity of information regarding Colibacillosis in broiler chickens in Kashmir, this study was undertaken to evaluate the pathological alterations on the structure and functionality of different organs of infected broiler chickens.

## Materials and Methods

### 1. Study area and study period

The study was conducted at Division of Veterinary Pathology, SKUAST-KASHMIR during the period of March to August, 2023.

### 2. Selection of the cases

Both organized and unorganized poultry farms were visited regularly during the study period in order to record the mortality. During this study period, 200 mortality in broiler chicken were recorded due to Colibacillosis at 10 poultry farms that were the focus of our investigation. The outbreaks suspected for *Escherichia coli* in broiler chicken were identified based on the history, clinical signs and lesions, after following a thorough post mortem examination of birds.

### 3. Hematological examinations of Sick birds

Blood was taken for serological and haematological screening from morbid birds exhibiting typical clinical signs of Colibacillosis. Blood was also taken for hematological analysis as a comparison from birds in normal flocks that appeared to be healthy and had no history of illness.

Ten birds with typical signs of Colibacillosis were randomly selected for blood collection from each farm. The blood was collected through wing vein and about two milliliters of blood were placed into sterile tubes and slants were made ready for the collection of serum for biochemical estimation. On the other hand, one milliliter of blood was placed into vials containing EDTA for haematological analysis of the affected birds. Same procedure was carried out for the birds which did not showed any apparent clinical signs and lesions. The estimation of hemoglobin (Hb), packed cell volume, total erythrocyte counts (TEC), and total leucocyte count (TLC) were among the hematological investigations in case of colibacillosis infected birds.

### 4. Serum Analysis

Various blood biochemical parameters were estimated by using Olympus biochemistry analyser with compatible kits from aspen chemicals. The parameters studied included.

1.	Total protein	4.	Albumin
2.	SGOT /AST	5.	Globulin
3.	SGPT/ALT	6.	Creatinine

### 5. Relative organ weight ratio

The lymphoid organs like spleen, thymus, caecal tonsils and Bursa of Fabricius were removed from dead birds that had

contracted Colibacillosis naturally. The relative weights of each of these organs were calculated using the method outlined by Garg (2004) [11].

### 6. Statistical analysis

All the data generated in the study was analysed using SPSS software as per Snedecor & Cochran, (1994) [26].

### 7. Pathoanatomical studies

The carcasses were subjected to a thorough and systematic necropsy for examining and recording of the lesions true to colibacillosis which included perihepatitis, pericarditis, omphalitis, tenosynovitis, airsacculitis and cellulitis. The representative samples of liver, heart, lung, air sacs, kidney, intestines and spleen were collected from colibacillosis, subsequently preserved and fixed in 10% buffered formalin for histopathological examination and processed by routine paraffin embedding technique as described by Luna, 1968.

### 8. Molecular Detection of *Escherichia coli* by Polymerase chain reaction

The DNA extraction from *E. coli* was carried by boiling and snap chill method. The primers utilized for the identification of *E. coli* targeted its 16S rRNA gene. The primers used were as per the method described by Sabat *et al.*, (2000) and the sequence is given in table-1. Polymerase chain reaction (PCR) was performed in a 25 µl total volume, including 12.5 µl of Go Taq®Green Master mix (2X) (Promega), 1 µl for each primer (Operon), 2 µl of DNA template, and 8.5 µl of Nuclease-free water. The PCR program for the amplification of the 16S rRNA gene included an initial denaturation cycle of 8 min at 95 °C, followed by 35 cycles, each consisting of 1 min at 94 °C, 1.5 min at 55 °C, and 1 min at 72 °C. A final extension step of 10 min at 72 °C was performed. The resulting amplicons were separated through gel electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized using a transilluminator with ultraviolet light. The PCR amplification products for each gene were compared with a 100bp DNA ladder (Thermo scientific).

## Results

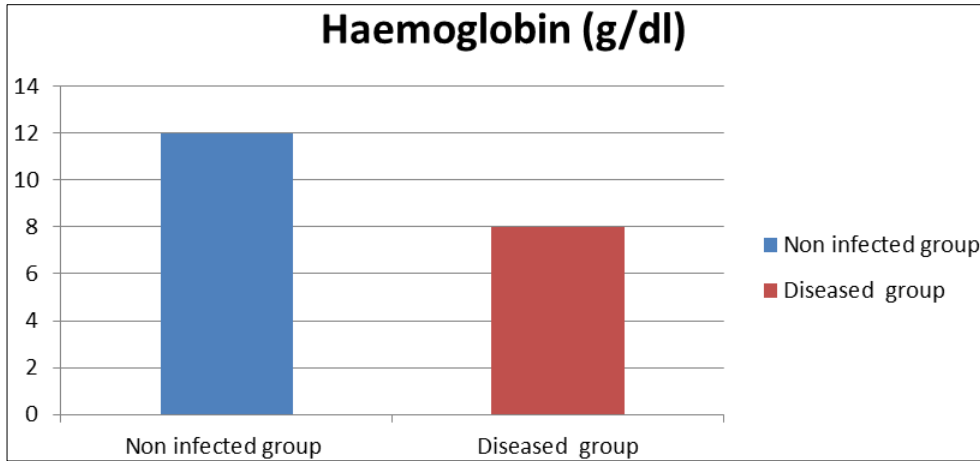
### 1. Hematology

The hematological findings revealed substantial differences in hemoglobin level, packed cell volume, total red blood cell (RBC) count and total white blood cell (WBC) count among the study groups in this study as shown in Table 1 & Table 2.

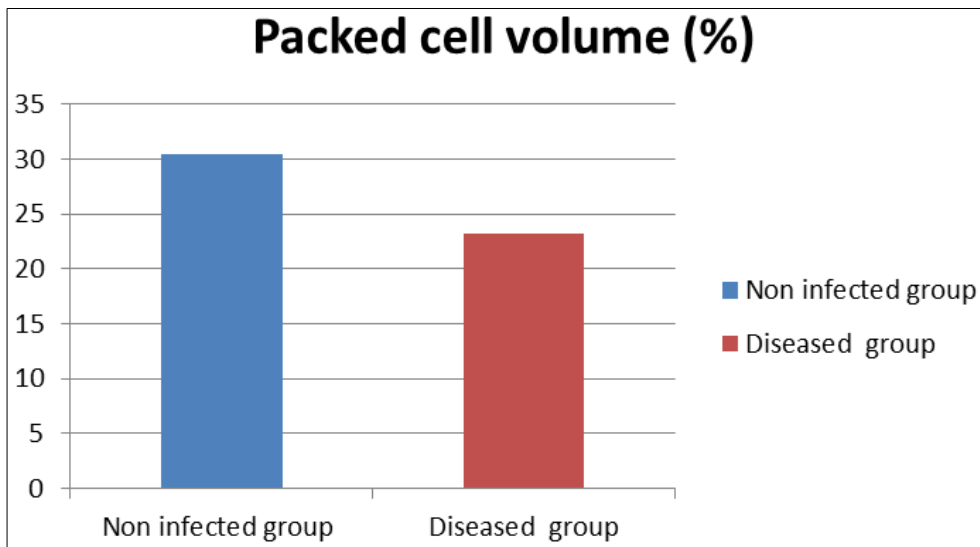
**Table 1:** Hematological parameters in different groups of Broiler Chickens

S. No.	Group	Hemoglobin Concentration (g/dl)	Packed cell volume (%)
1.	Non infected group	12.00±0.68 <sup>a</sup>	30.45±1.53 <sup>c</sup>
2.	Diseased group	8.00±0.58 <sup>b</sup>	23.26±1.47 <sup>d</sup>

Means of various Hematological parameters differ significantly.



**Fig 1A:** Haemoglobin alteration (Hb) in Diseased and Non Diseased Broiler Chickens

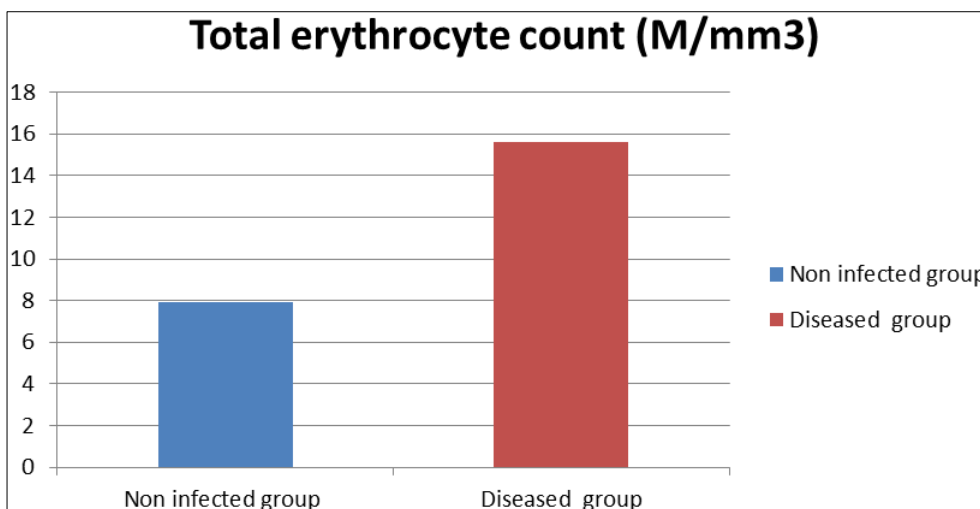


**Fig 1B:** Packed cell volume alteration (PCV) in diseased and non-diseased in broiler chickens

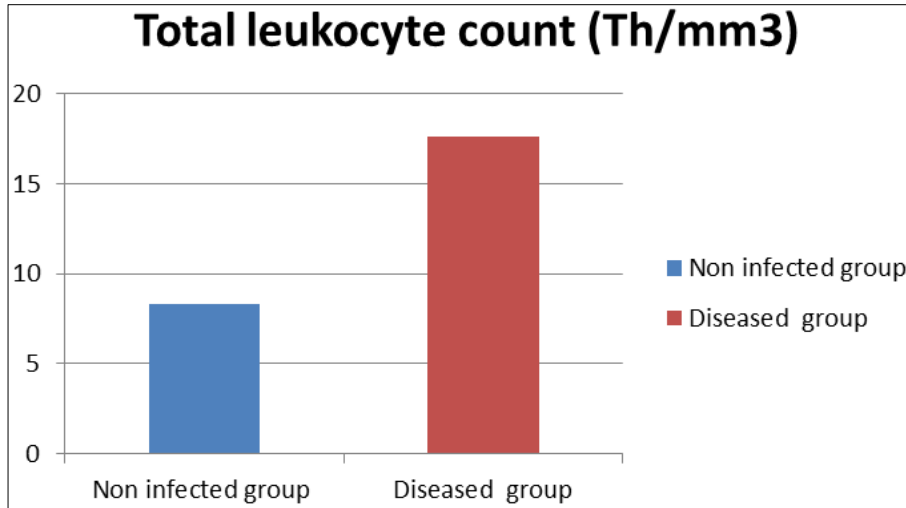
**Table 2:** Hematological parameters in different groups of Broiler Chickens

S. No.	Group	Total erythrocyte count (M/mm <sup>3</sup> )	Total leukocyte count (Th/mm <sup>3</sup> )
1.	Non infected group	7.90±0.12 <sup>a</sup>	8.30±0.25 <sup>c</sup>
2.	Diseased group	15.62±1.42 <sup>b</sup>	17.62±1.52 <sup>d</sup>

Means of various Hematological parameters differ significantly



**Fig 2A:** Total erythrocyte count (TEC) alterations in Colibacillosis in Broilers



**Fig 2B:** Total leukocyte count (TLC) alterations in Colibacillosis in Broilers

**2. Biochemical Analysis**

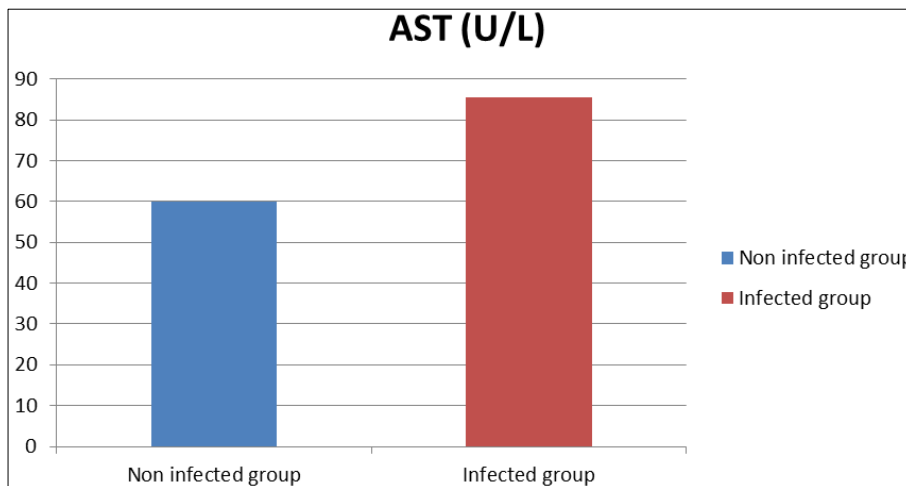
The results of biochemical alterations have been presented in Table 3. In comparison to non-infected chickens, the mean AST and ALT values of chickens infected with *Escherichia coli* were considerably higher. The overall mean of total protein, albumin and globulin value in *Escherichia coli* infected chicken was significantly lower when compared with non-infected chicken. The decrease in

total protein, albumin and globulin might be due to severe hepatic damage found in Colibacillosis

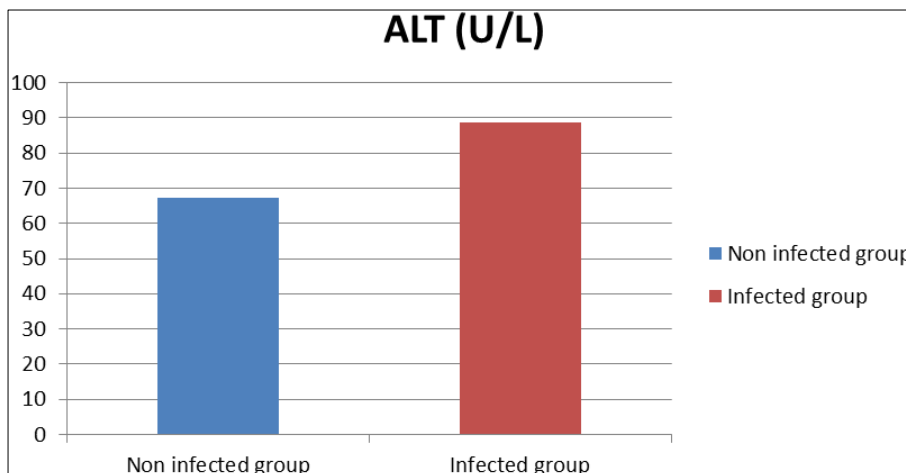
**Table 3:** Serum biochemical alterations in broiler chicken affected with Colibacillosis

S. No.	Group	AST (U/L)	ALT (U/L)
1.	Non infected group	60.03±1.37 <sup>a</sup>	67.37±1.27 <sup>c</sup>
2.	Infected group	85.53± 3.52 <sup>b</sup>	88.72± 2.26 <sup>d</sup>

Means of various Hematological parameters differ significantly



**Fig 3A:** AST (U/L) level alteration in *E. coli* infected Broiler Chickens

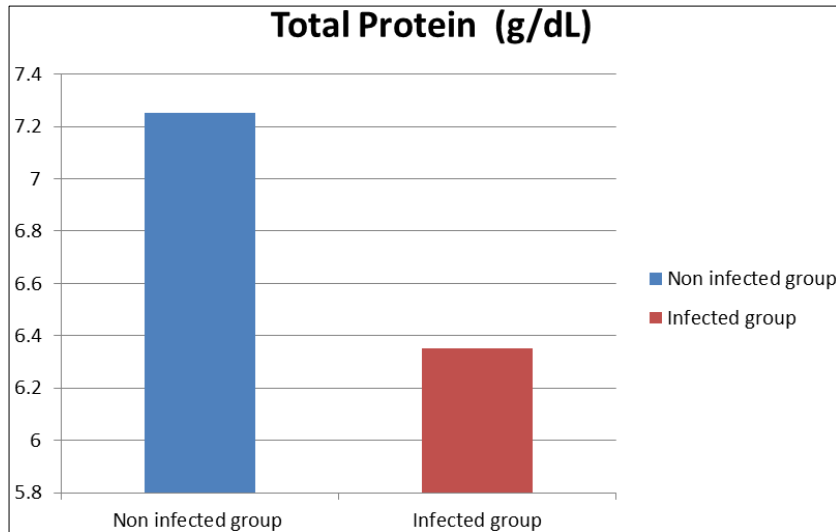


**Fig 3B:** ALT (U/L) level alteration in *E. coli* infected Broiler Chickens

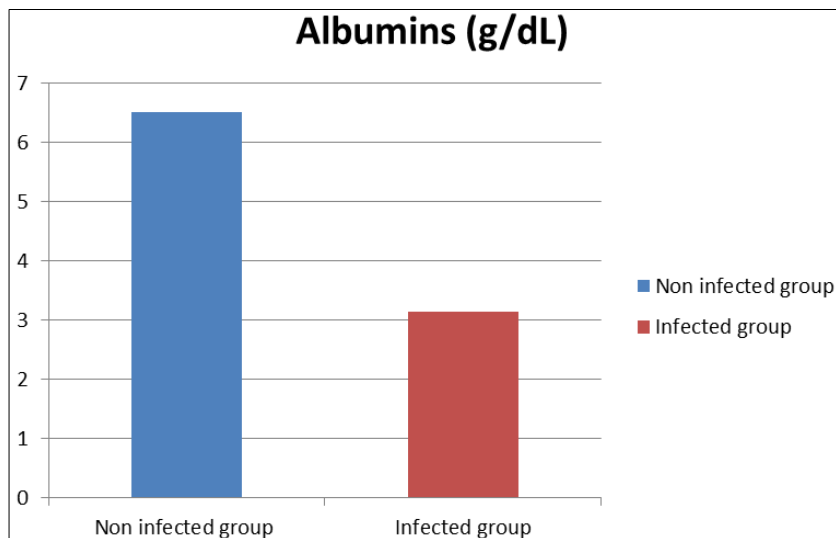
**Table 4:** Serum biochemical alterations in broiler chicken affected with colibacillosis

S. No.	Group	Total Protein (g/dL)	Albumins (g/dL)	Globulins (g/dL)
1.	Non infected group	7.25 ±0.04 <sup>a</sup>	6.52± 0.06 <sup>c</sup>	3.64±0.04 <sup>e</sup>
2.	Infected group	6.35±0.07 <sup>b</sup>	3.15± 0.01 <sup>d</sup>	1.75± 0.05 <sup>f</sup>

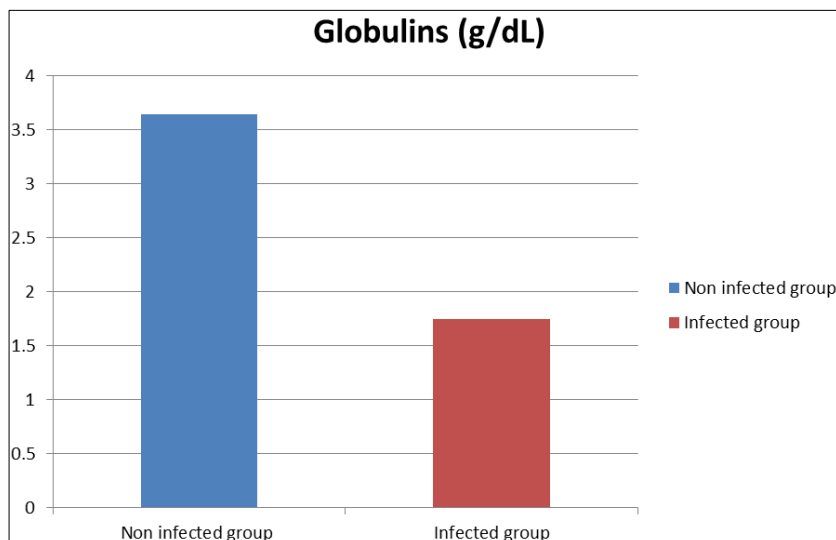
Means of various biochemical parameters differ significantly



**Fig 4A:** Total Protein (g/dL) level alteration in *E. coli* Infected Broiler Chickens



**Fig 4B:** Albumins (g/dL) level alteration in *E. coli* infected Broiler Chickens

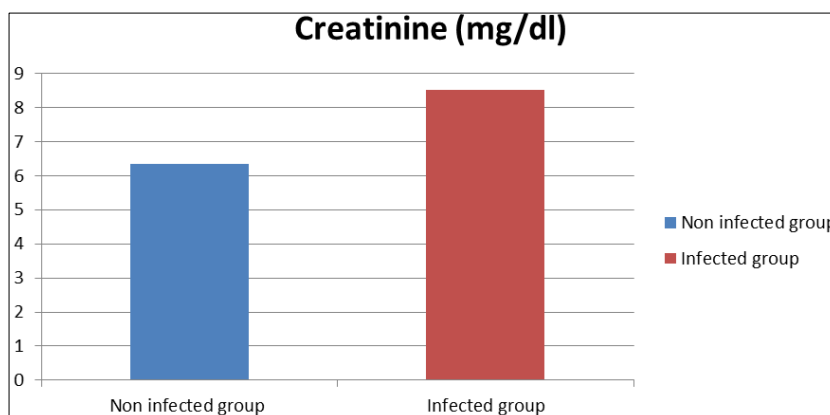


**Fig 4C:** Globulins (g/dL) level alteration in *E. coli* infected Broiler Chickens

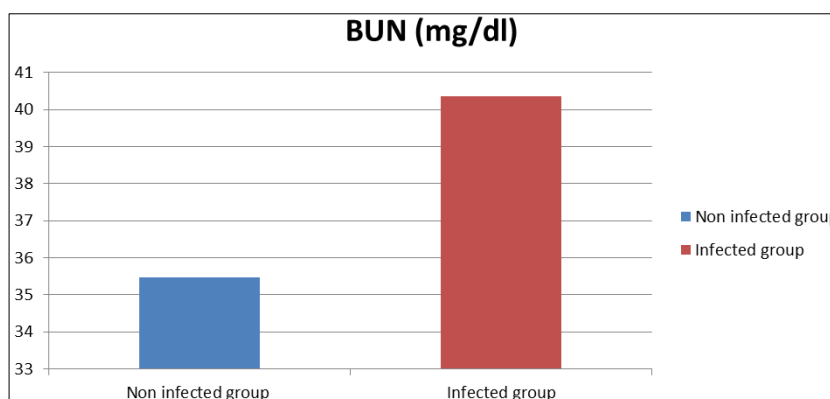
**Table 5:** Serum biochemical alterations in broiler chicken affected with colibacillosis

S. No.	Group	Creatinine (mg/dl)	BUN (mg/dl)
1.	Non infected group	6.35±0.27 <sup>a</sup>	35.47±1.65 <sup>c</sup>
2.	Infected group	8.53±0.18 <sup>b</sup>	40.35±1.71 <sup>d</sup>

Means of various biochemical parameters differ significantly.



**Fig 5A:** Creatinine (mg/dl) level alteration in *E. coli* infected Broiler Chickens



**Fig 5B:** BUN (mg/dl) level alteration in *E. coli* infected Broiler Chickens

**(D) Relative organ weight ratio**

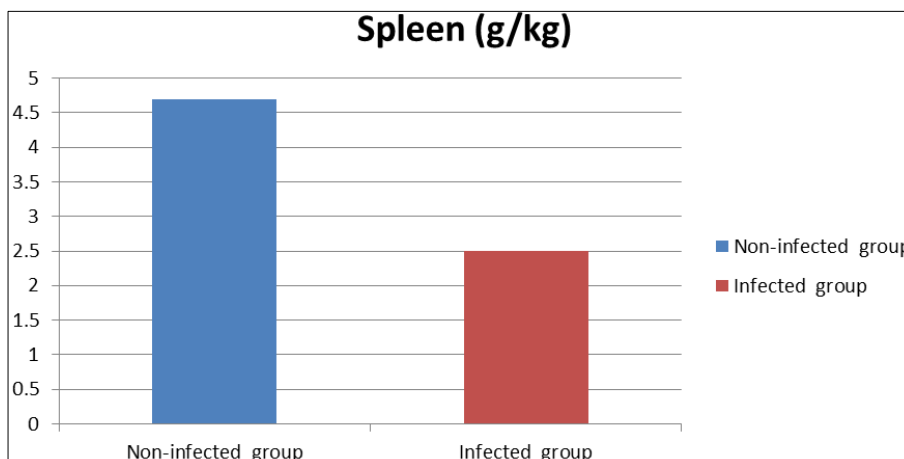
The mean weights of spleen, bursa, thymus and caecal tonsils from both infected and non-infected birds were comparable. On the other hand, when comparing the mean weights of various lymphoid organs in Colibacillosis-

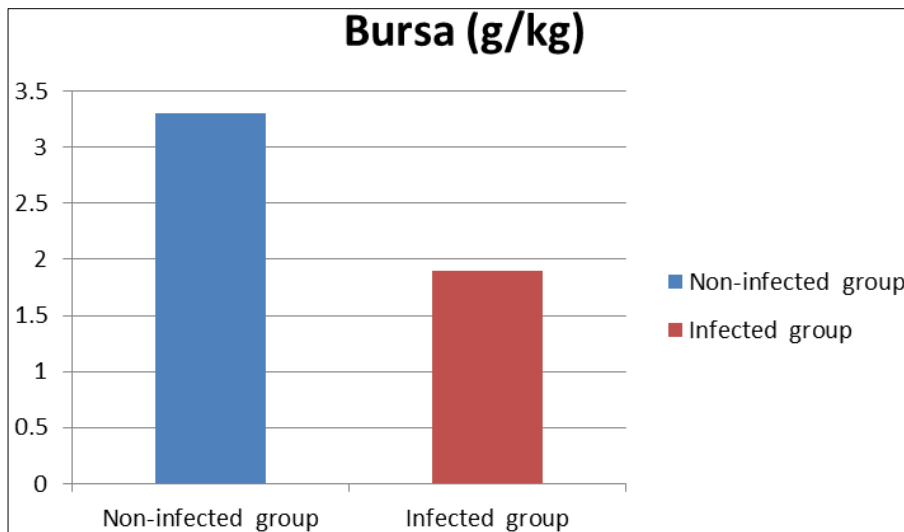
infected birds to those of normal birds, a significant ( $p \leq 0.05$ ) decrease was observed in the weight of lymphoid organs in *E. coli* infected bird. The decrease in the weight of the lymphoid organs must be due to the lymphoid depletion.

**Table 6:** Relative weight of lymphoid organs of broiler chickens infected with Colibacillosis

Groups	Spleen (g/kg)	Bursa (g/kg)
Non-infected group	4.7 <sup>a</sup> ± 0.13	3.30 <sup>c</sup> ± 0.35
Infected group	2.50 <sup>b</sup> ± 0.25	1.90 <sup>d</sup> ± 0.13

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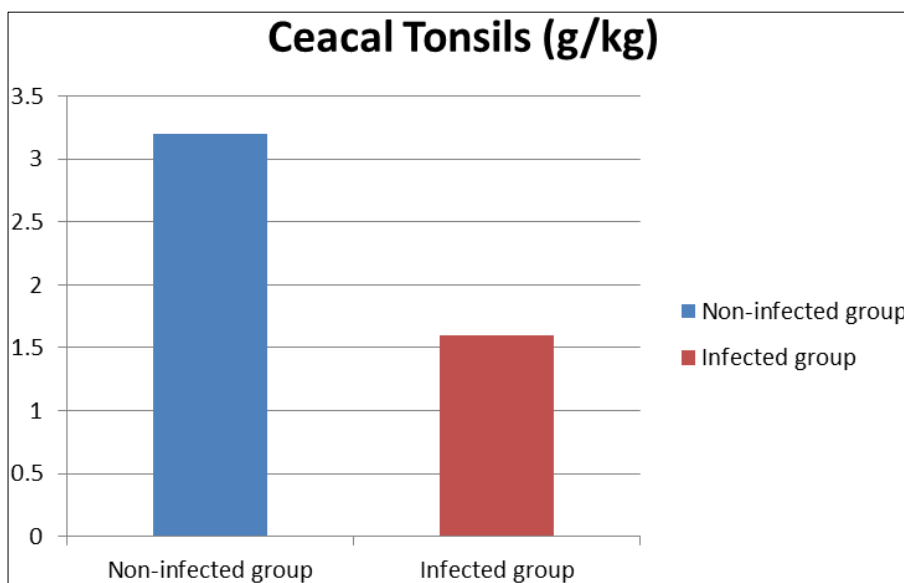
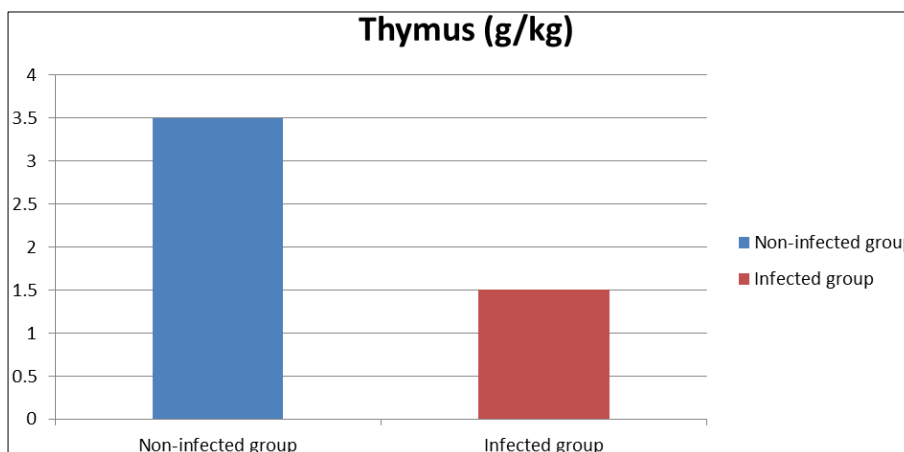


**Fig 6A:** Relative weight of Spleen and Bursa in Infected and Non Infected Broiler Chickens

**Table 7:** Relative weight of lymphoid organs namely Thymus and Caecal tonsils of broiler chickens infected with colibacillosis

Groups	Thymus (g/kg)	Caecal Tonsils (g/kg)
Non-infected group	3.5 <sup>a</sup> ± 0.17	3.2 <sup>c</sup> ± 0.27
Infected group	1.5 <sup>b</sup> ± 0.29	1.6 <sup>d</sup> ± 0.35

Means in different columns bearing common superscript does not differ significantly



**Fig 7A:** Relative weight of Thymus and Caecal Tonsils in Infected and Non Infected Broiler Chickens

The graph represents alteration in relative weight of thymus and caecal tonsils in infected group and Non-infected group

### Clinical Signs

Birds infected with *E. coli* appeared weak, depressed and exhibit less activity as compared to normal birds. The *E. coli* affected birds shows reduced feeding ability and may have mucoid diarrhea leading to the dehydration with sunken eyes, dry skin and increased thirst. The respiratory symptoms such as coughing, sneezing, nasal discharge, labored breathing and dyspnea can be seen in severe *E. coli* cases. The *E. coli* infection in case of in laying hens can lead to a decrease in egg production and the production of misshapen eggs. Some cases of *E. coli* infection in birds can cause swollen joints leading to the lameness and difficulty in walking. The *E. coli* infection in birds can have severe consequences-like high mortality and morbidity rates, particularly in young birds resulting to the huge economic losses to the poor farmers. The birds with *E. coli* infections may also have reduced body weight, increased thirst, ruffled plumage and disheveled feathers. The infected breeding flocks may experience reduced fertility and may have substantial mortality rates, especially in young chicks as compared to adult birds. Birds infected with *E. coli* may also exhibit neurological symptoms as paralysis, convulsions, and tremors. Some poultry flocks showed symptoms of disoriented movements, like stumbling and having trouble walking. The infected birds also tend to sit with drooping wings and have a hunched posture. The affected poultry birds may huddle up to stay warm. The infected birds at the end of study period may reveal pale mucus membranes with other different clinical manifestations like Tibialdyschondroplasia, aberrant bending of the tibial bones, enlarged hock joints, sternal recumbency, convulsion, eye lacrimation, excessive salivation, restlessness and ataxia. The keel bone appeared sharp and pointed due to emaciation. The infected birds may show significant increase in temperature beginning from the 1<sup>st</sup> day till end of the study period. The diseased birds also reveal stunted growth, watery droppings, weak muscles and a loss of reaction to stimuli before they died. The affected birds may reveal puffed feathers, closed eyes, stood motionless for long period of time. The bird's mouth appeared to be clogged with mucus and may also show repeated sneezing. Death usually resulted from respiratory failure. Before death, the birds fell suddenly revealing uneasiness, paddling of legs and unconsciousness.

### Pathomorphology

#### Heart

##### Gross Pathology

Grossly, the heart showed signs of congestion and was covered with thick fibrinous layer in severe *E. coli* cases. The congestion in the heart results into fluid accumulation leading to edema. The heart of the affected birds appeared swollen and has a pale appearance.

##### Histopathology

The pericardium was generally thickened and engorged with blood. In mild cases, heterophilic infiltration and fibrinous exudate results in the thickening of the pericardium. In addition to this, the histopathological analysis revealed also fibrinous pericarditis with congested blood vessel (Fig 1). In severe cases, there was an excessive thickening of the

pericardium with necrotic regions and infiltration of heterophils. The microscopic examination further revealed severe myopathy, disruption of the heart muscle (Fig 2). and extensive leucocytic infiltration, primarily of heterophils visible in the spaces between the muscle fibers.

#### Liver

##### Gross Pathology

The liver of the affected birds in moderate cases revealed deposition of fibrin on their surface. In severe cases, a thick layer of fibrin was covered the whole liver, giving it a "bread and butter" appearance. Some of these livers had a dark discoloration, appeared friable and had localized regions of necrosis visible on the surface.

##### Histopathology

The microscopic examination of the liver of infected birds revealed degeneration in the form of cellular swelling, individualization of hepatocytes, deformed hepatic cords, congested blood vessel and Fibrosis (Fig-3). In some severe cases, kupfer cell hyperplasia and thickened liver capsule characterized by the presence of a significant amount of fibrin, Sinusoidal congestion and infiltration of several heterophils in the affected tissue section (Fig 4).

#### Kidney

##### Gross Pathology

In most Colibacillosis cases, the kidneys were enlarged, swollen and edematous in nature. On the surface of the kidneys, necrotic foci and pin-point hemorrhages were also found in severe *E. coli* cases. At the end of the study period, the kidneys of affected birds were mottled with pale discoloration.

##### Histopathology

The microscopic examination of the affected kidneys revealed congestion and hemorrhage in interstitial tissue accompanied with degeneration of renal tubules (Fig 5). In severe cases, the kidneys of infected birds revealed atrophy of the glomerulus and denudation of renal tubular epithelium along with focal infiltration of leucocytes consisting of both mononuclear cells and heterophils (Fig 6).

#### Lung

##### Gross Pathology

The Pulmonary lesions ranged in severity from congestion, oedema to lung consolidation. Some of the severe *E. coli* cases revealed patchy to diffuse areas of lung consolidation. In most instances, the consolidation was unilateral and in more advanced cases it was bilateral.

##### Histopathology

Generally, the lung revealed parabronchial hemorrhages and interlobular septal congestion. The inflammatory cells like heterophils and mononuclear cells were found infiltrating the primary and the tertiary bronchioles lumen. There have been instances of acute bronchopneumonic changes, which were characterized by the infiltration of leucocytes and the presence of exudates in the bronchioles and parabronchi (Fig-7). The microscopic analysis of the lung further revealed thickened wall of the congested blood vessel as a result of inflammatory cells (Fig-8).



**Air sacs****Gross Pathology**

The air sacs had a thin film of fibrin covering them in mild cases and a thick layer in advanced stages characterized by caseous exudates on the surface. The lesions were more pronounced on the thoracic air sacs as compared to the abdominal air sacs.

**Histopathology**

The microscopic examination of the air sacs membrane revealed increased thickness in each case due to a leucocytic infiltration consisting predominantly of heterophils. The histopathological examination of the air sacs further revealed that the large amounts of fibrinous exudate and oedema were commonly pathological changes observed in the affected air sacs.

**Bursa****Gross Pathology**

In most cases of *E. coli* infection, the affected bursa of Fabricius showed lesions of edema, enlargement and swelling. The bursa of the affected poultry flocks were hemorrhagic with blood and cheesy exudate mixed together in the lumen. In addition to this, bursa of the affected birds was friable in nature with excessive amount of exudate and fibrin deposits in the bursal cavity.

**Histopathology**

The histopathological examination of the bursa of Fabricius revealed lymphoid depletion, which is characterized by a significant reduction in the quantity of lymphocytes within bursal follicles and the consequent loss of the bursal architecture (Fig-9). The further microscopic analysis revealed interstitial oedema, congestion, degeneration, atrophy and necrosis of the bursal follicles (Fig-10). As the disease progresses, the bursa undergoes further degeneration and the remaining lymphoid tissue may be replaced by connective tissue.

**Thymus****Gross Pathology**

Grossly, the thymus appeared smaller as compared to normal one, which could be the result of the *E. coli* infection-induced thymic atrophy and lymphoid depletion. The majority of the severe cases of Colibacillosis were found to have swelling, congestion and edema upon gross examination. In few *E. coli* cases, the thymus may become soft and fragile in nature.

**Histopathology**

The histopathological analysis of the thymus showed depletion of lymphoid tissue resulting in the decreased quantity of lymphocytes and thymic atrophy (Fig-11). The lymphocyte depletion impairs the normal bird's immune system capacity to build a successful defense against *E. coli* infections. The microscopic examination of the thymus also showed infiltration of inflammatory cell, such as heterophils, macrophages and lymphocytes in response to the *E. coli* infection. In severe cases, microscopic examination further revealed degeneration and necrosis inside the thymus (Fig-12). These necrotic areas show that

the bacterial cytopathic effects have resulted in localized cell death.

**Spleen****Gross Pathology**

The spleen was enlarged and congested in the most of the *E. coli* cases. The scattered necrotic foci were also found on the spleen's surface in a few *E. coli* affected cases.

**Histopathology**

The microscopic examination revealed severe splenic congestion, hemorrhages, lymphoid depletion (Fig-13) and many necrotic foci were also evident in the affected tissue section (Fig-14). In most of the severe cases, secondary germinal centers were formed as a result of extensive necrosis in the spleen's primary follicles. The histopathological examination of the spleen further revealed perivascular infiltration with thickening of the wall of the blood vessel.

**Intestine****Gross Pathology**

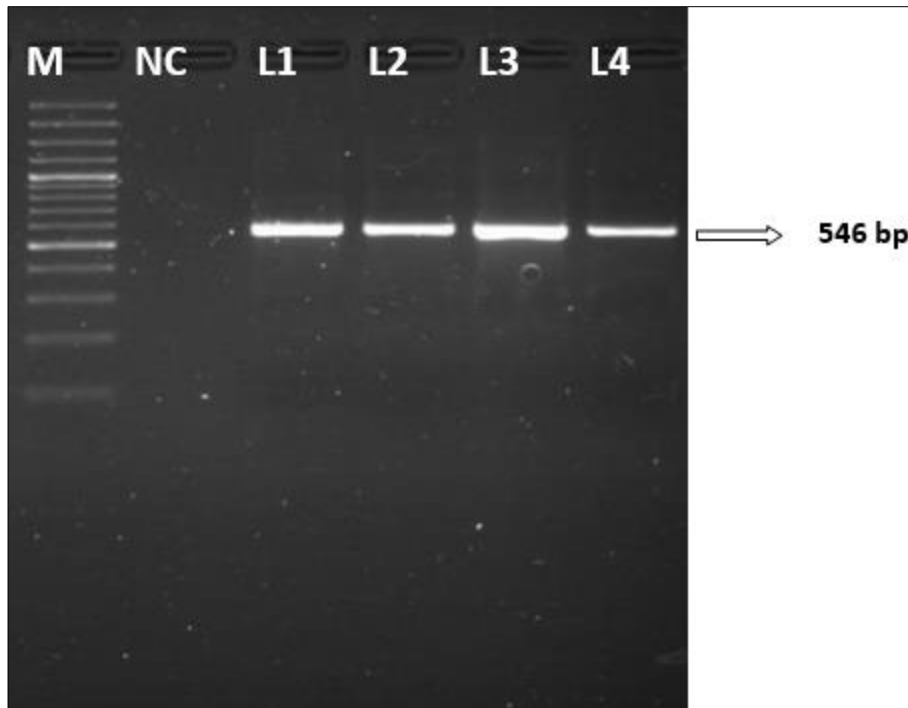
The affected intestine has more vascularity and appears redder than normal because of vascular congestion. The gross examination of the intestine also reveals large hemorrhagic patches and pinpoint necrotic spots within the intestinal wall as a result of the hemorrhages in the affected intestinal mucosa. The *E. coli* infection can induce inflammation that thickens the intestinal wall and causes edema, which gives the appearance of swollen intestines. The affected intestinal mucosa consists of fibrin and debris which may appear as yellowish plaques. Sometimes narrowing of the intestinal lumen caused by fibrosis may also be observed in the affected intestine which can be resulted from inflammation and ulceration.

**Histopathology**

The microscopic examination of the intestinal mucosa revealed degeneration, denudation and necrosis. The affected intestinal villi showed blunting of the villus due to the presence of inflammatory cells (Fig-15). The microscopic examination further revealed glandular atrophy and fragmentation of intestinal villi (Fig-16). The microscopic analysis of ileum tissue section revealing denudation of tunica serosa with villous blunting (Fig-17). The histopathological examination of caecum revealing thickening of tunica muscularis with atrophy of the intestinal glands (Fig-18). The inflammatory cells like heterophils and lymphocytes can penetrate the intestinal lamina propria, intestinal glands and intestinal epithelium. The normal architecture of the intestinal epithelium may be disturbed by these heterophils.

**Molecular Detection by Polymerase Chain Reaction**

A total number of 200 suspected broiler chicken samples were screened and among the isolates recovered 200 were identified as *E. coli* by PCR. The resulting amplification product had a length of 546 base pairs (bp). In this study, all the 200 isolated colonies showed amplification of 546 bp of 16SrRNA gene by Polymerase Chain Reaction (Fig 8a).



M is 100bp DNA Ladder plus, NC= Negative control (*Staphylococcus aureus*). L1= *E. coli* (ATCC 25922), L2-L4= *E. coli* isolates

**Fig 8a:** Detection of 16SrRNA gene by Polymerase Chain Reaction

## Discussion

The rearing of domesticated birds, such as chickens, ducks, turkeys, and geese, with the intention of generating meat, eggs, and other poultry products, is known as poultry farming (Singh *et al.*, 2018) [22]. It is an important sector of the agriculture and is essential to supplying animal protein to the world's population. Initially, poultry farming was primarily focused on egg production, but now it has been expanded to meat production as well. With a variety of production methods and management techniques, poultry farming is a highly specialized and productive industry. Poultry farming has several benefits, chief among them being its high productivity and short production cycle in comparison to other animals. Poultry farming has a significant economic impact, providing employment opportunities, income generation and food security.

The purpose of this experiment was to evaluate the pathomorphological and clinical alterations observed in the Colibacillosis infected broiler chickens. The clinical signs like depression, watery diarrhea, dehydration, ruffled feather, trembling, gasping and emaciation were recorded in *E. coli* infection (Gangane *et al.*, 2006 and Shah *et al.*, 2019) [10, 23]. The hematological parameters like Hb, PCV, TEC and TLC in the affected broiler chickens were recorded altered as compared to normal values (Umar *et al.*, 2016 and Christie *et al.*, 1999) [27, 6].

Myocarditis may be the reason of the increase in AST activity in birds infected with *Escherichia coli* (Benjamin *et al.* 1998) [5], whereas the release of this enzyme from the liver as a result of hepatitis is likely the origin of the increase in ALT. Since an increase in activity of serum ALT is taken into consideration to be an indicator of hepatic cellular damage and alteration in permeability of hepatic mobile membrane (Christen *et al.*, 1996) [7]. The earlier reports also showed similar pattern of increase in AST and ALT levels in *E. coli* infected birds as reported by various scientists (Christie *et al.*, 1994 [8], Jindal *et al.*, 2003 and Koynarski *et al.*, (2010) [12, 14]. The decrease in albumin and total protein levels might be related to severe hepatic

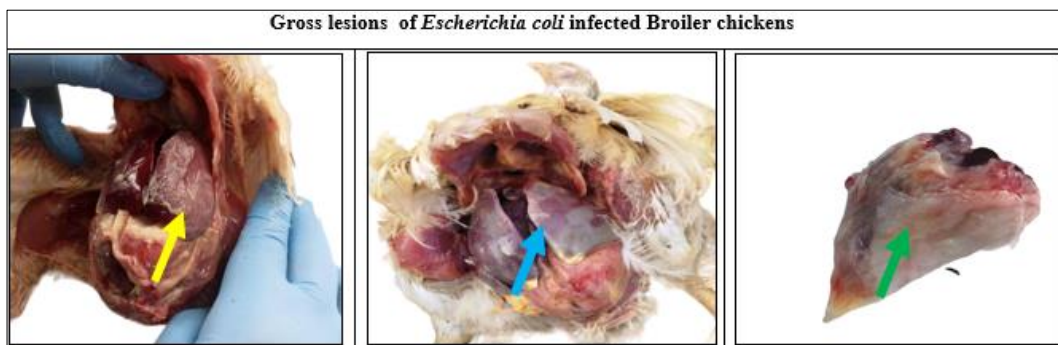
damage, as observed in cases of Colibacillosis. These findings are consistent with reports from multiple scientific studies (Jindal *et al.*, 2003) [12], which also found significant drops in albumin, globulin, and overall protein levels in birds infected with *Escherichia coli*. In this investigation, blood urea nitrogen and creatinine levels significantly increased in all of the *E. coli* infected birds. The increased blood urea nitrogen and creatinine levels might be due to the pathomorphological alterations in the kidneys of the infected birds (Sharma *et al.*, 2015) [24].

A significant reduction in relative weight of lymphoid organs were observed in the infected birds when compared with the weight index of lymphoid organs of normal birds (Berthault *et al.*, 2018) [3]. The severe atrophy of the lymphoid organs and lymphocyte depletion in the lymphoid follicles of the bursa, spleen, thymus, and caecal tonsils could be the cause of weight loss.

The liver had extensive gross pathological lesions, including adhesions, rounding of the margins, congestion, and necrotic foci on the liver's surface. Microscopically, there might be the deposition of fibrin on the capsular wall heterophilic infiltration and kupfer cell hyperplasia (Kumar *et al.*, 2013 and Renu *et al.*, 2012) [13, 21]. An examination of the heart grossly revealed adhesions between the heart and the chest cavity, congestion, and varying fibrinous layer deposition on the pericardium. Cardiomegaly, involving both heart chambers, was observed during the gross examination of the heart. The microscopic examination revealed mononuclear cellular infiltration and fibrinous exudates in the affected heart tissue sections. These types of lesions have been previously described by few researchers in a natural outbreak of colibacillosis in broiler chickens (Nakamura *et al.*, 1985, Gangane *et al.*, 2006) [20, 10] and Kumar *et al.*, 2013) [13]. Grossly the lungs revealed congestion and consolidation. The microscopic examination of the affected lungs revealed pneumonic changes as evident with infiltration of inflammatory cell in the lung tissue section with thickening of the wall of the blood vessel (Renu *et al.*, 2012) [21]. In most cases, kidney lesions ranged from oedema

and congestion to the development of tiny hemorrhages on the surface. The other pathological alterations like Congestion, hemorrhage, renal tubule degeneration, and localized infiltration of leucocytes of both mononuclear cells and heterophils were also observed in the damaged kidney tissue under microscopic analysis of the kidneys. These findings are in accordance with the results of other researchers (Baliar Singh *et al.*, 1993) <sup>[1]</sup>, who also observed kidney oedema and congestion in experimental colibacillosis (Gangane 2006 and Kumar *et al.*, 2004) <sup>[10, 15]</sup>. The fibrinous deposition on air-sacs could be attributed to severity of outbreak and an important pathological lesion in colibacillosis in broiler chickens (Renu *et al.*, 2012) <sup>[21]</sup>. At the microscopic level, the primary and tertiary bronchioles' lumen revealed the presence of hemorrhages and congestion along with the infiltration of mononuclear cells and heterophils. Similar type of lesions has been observed in various Colibacillosis cases in broiler chickens which includes congestion, oedema and pneumonic foci (Gangane *et al.*, 2006, Tottori *et al.*, 1997 and Kumar *et al.*, 2013) <sup>[10]</sup>.

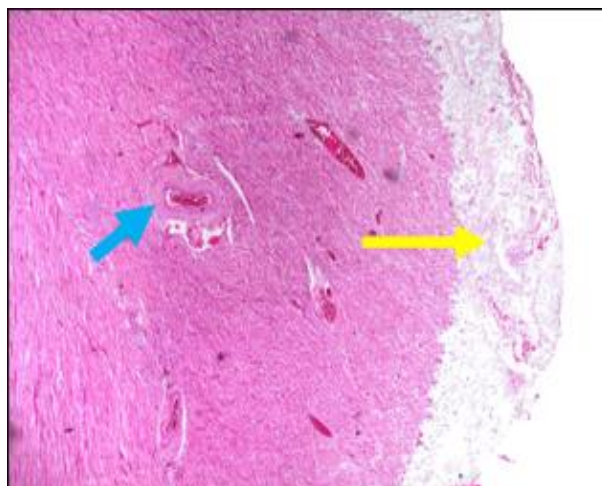
<sup>[13]</sup>. The pathological lesions like organ atrophy and hemorrhagic lesions were visible on the surface of the bursa and other lymphoid organs. The microscopic examination of the lymphoid organs like spleen, bursa, caecal tonsils, and thymus, revealed lymphoid depletion leading to the immune suppression in the birds (Shah *et al.*, 2019) <sup>[23]</sup>. In severe cases, a gross examination of the spleen revealed congestion and the presence of necrotic foci on the surface. The microscopic examination of the spleen showed lymphoid depletion, significant hemorrhages and congestion along with thickening of the blood vessel wall. The observations were in concurrence with the findings of various researchers published in reputed journals (Nakamura *et al.*, 1985) <sup>[20]</sup> and Kumar *et al.*, 2013) <sup>[15]</sup>. Tissue samples collected from different flocks were confirmed as *E. coli* infection by PCR assay. The amplification of 16SrRNA gene of *E. coli* revealed 546 bp products for ten different flocks. These findings are in accordance with previous researcher, who detected *E. coli* from broiler chickens by Polymerase Chain Reaction.



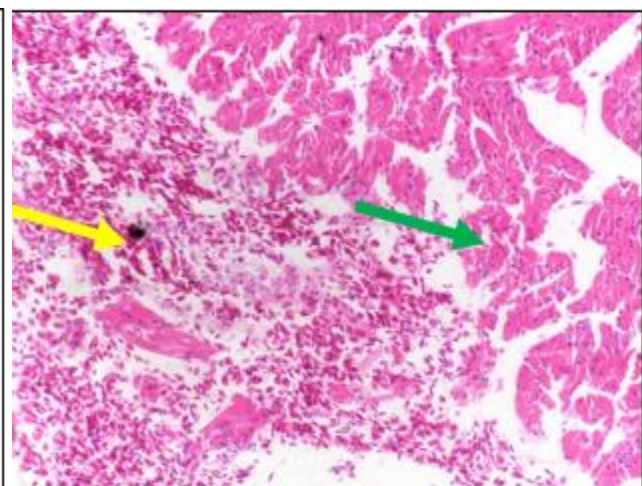
**Fig 9A:** Carcass of a broiler chicken showing Fibrinous perihepatitis and pericarditis



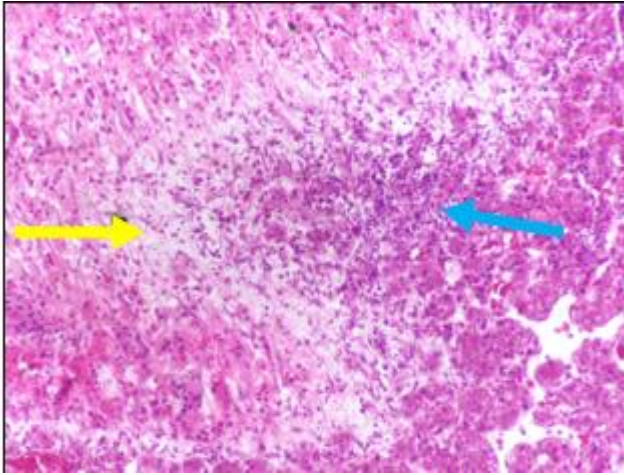
**Fig 9B:** Carcass of a broiler chicken showing Cloudy airsacs, Air sacculitis and peritonitis



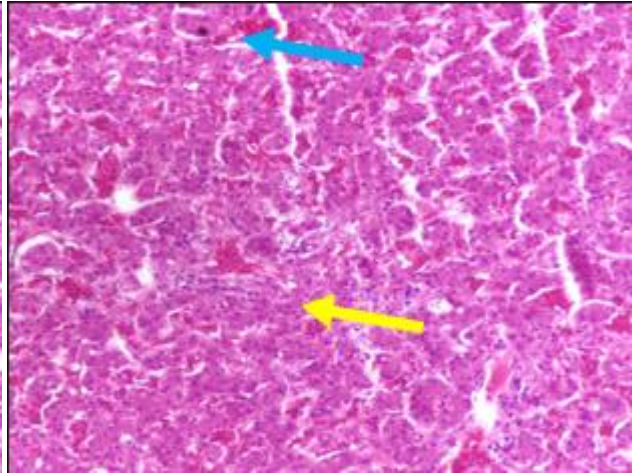
**Fig 1:** Photomicrograph of the heart revealing fibrinous pericarditis with congested blood vessel



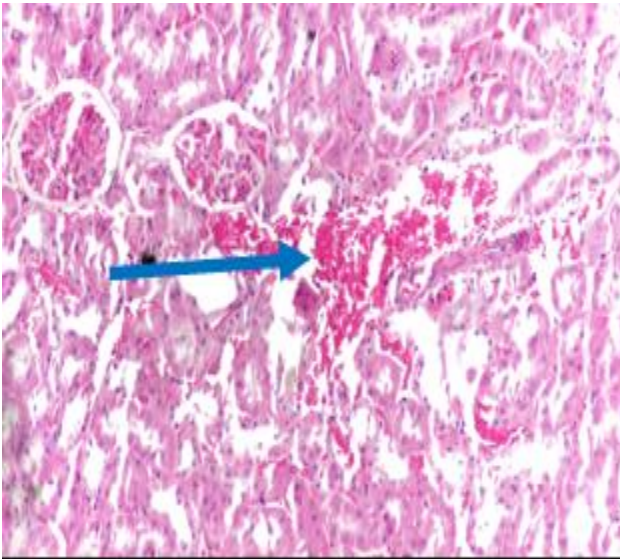
**Fig 2:** Photomicrograph of the heart tissue section revealing hemorrhage with disruption of muscle fibers



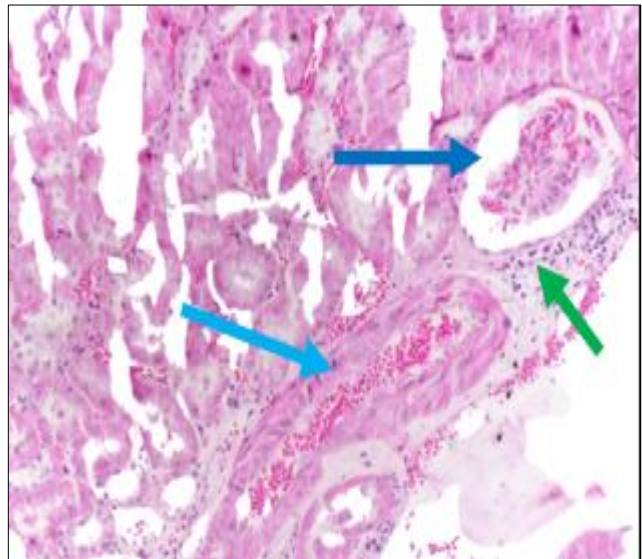
**Fig 3:** Photomicrograph of liver revealing fibrosis of the affected tissue with heterophilic infiltration



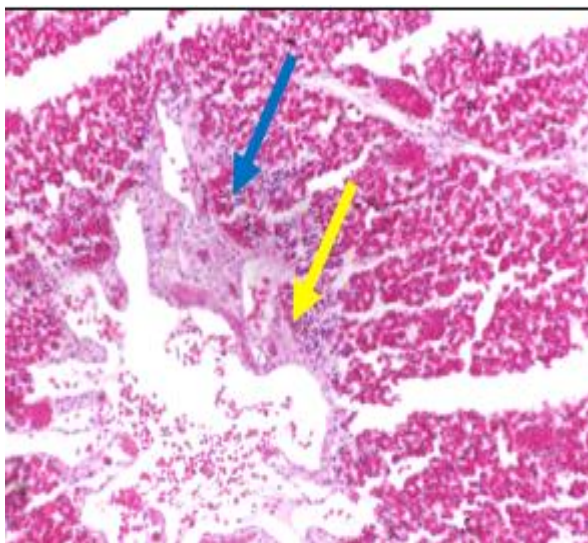
**Fig 4:** Photomicrograph of liver revealing sinusoidal congestion, cellular infiltration and degeneration



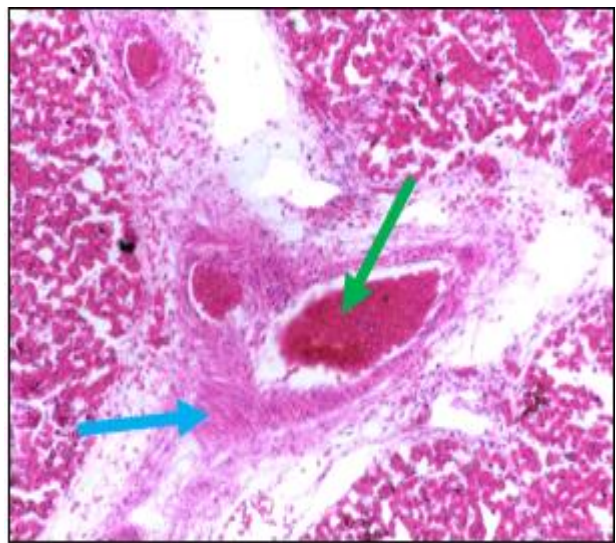
**Fig 5:** Photomicrograph of kidney revealing severe hemorrhage with tubular degeneration



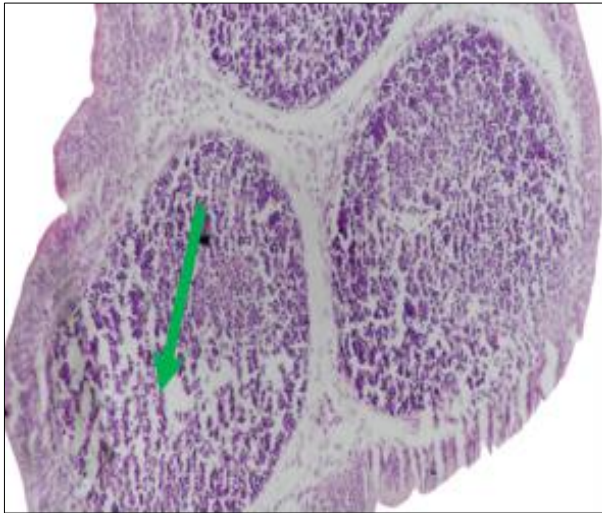
**Fig 6:** Photomicrograph of kidney revealing cellular infiltration, thick walled congested blood vessel with increased Bowman's space indicating glomerular atrophy



**Fig 7:** Photomicrograph revealing pneumonic changes in the lungs as evident with infiltration of inflammatory cell in the lung tissue section



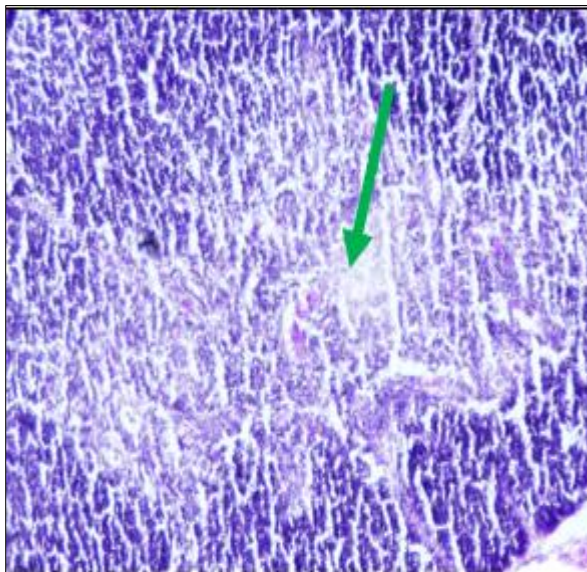
**Fig 8:** Photomicrograph of lung revealing thickened wall of the congested blood vessel as a result of inflammatory cells



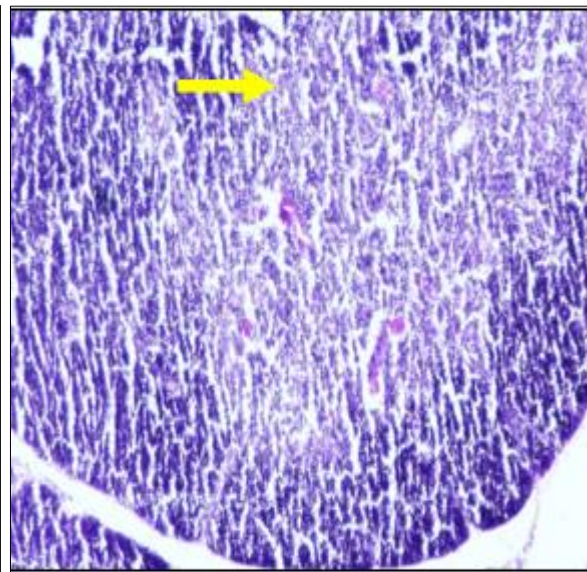
**Fig 9:** Photomicrograph of bursa revealing lymphoid depletion from bursa follicles



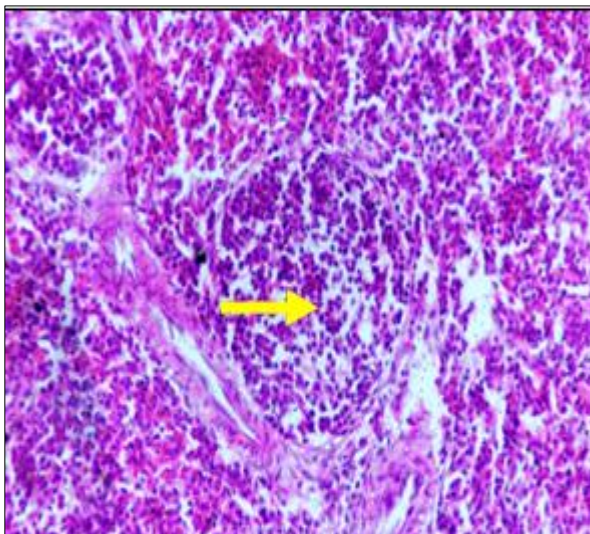
**Fig 10:** Photomicrograph of bursa revealing necrotic changes in the lymphoid follicles



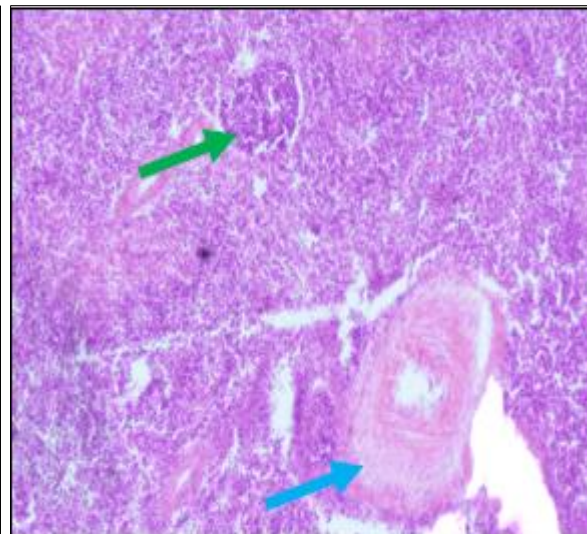
**Fig 11:** Photomicrograph of Thymus revealing lymphoid depletion from the lymphoid follicles



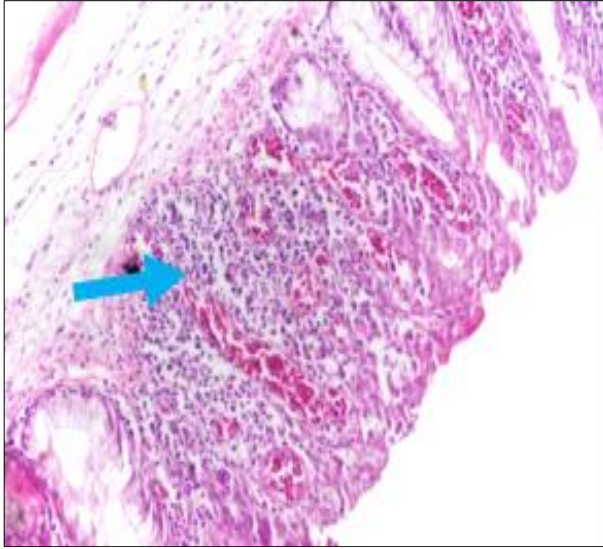
**Fig 12:** Photomicrograph of Thymus revealing necrosis within the thymic follicles



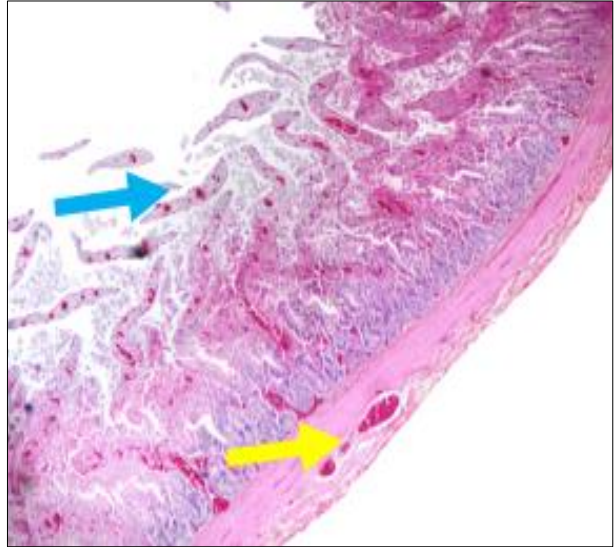
**Fig 13:** Photomicrograph of spleen revealing degeneration and destroyed lymphoid tissue in the white pulp



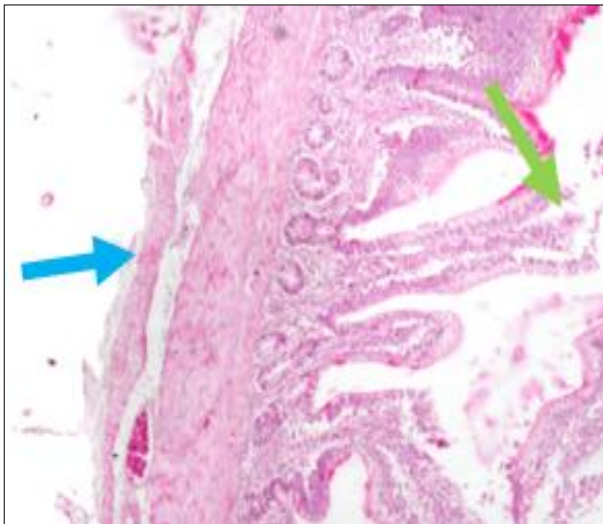
**Fig 14:** Photomicrograph revealing splenic atrophy with thickening of the wall of blood vessel



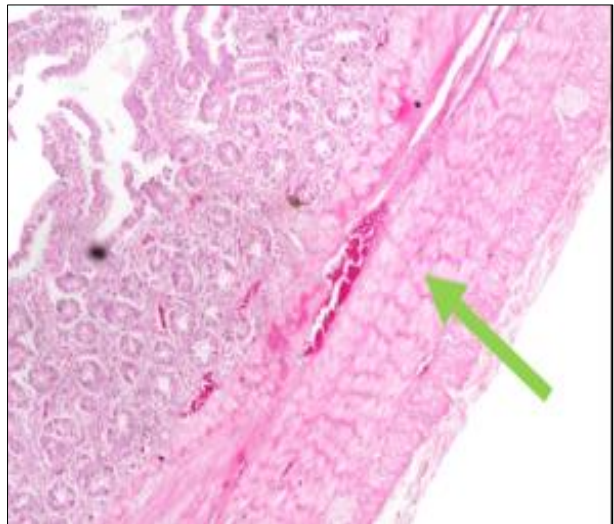
**Fig 15:** Photomicrograph of caecum revealing enteritis as evident with severe infiltration of heterophils in the affected tissue section



**Fig 16:** Photomicrograph of Jejunum revealing severe congestion with fragmentation of villi



**Fig 17:** Photomicrograph of ileum revealing denudation of tunica serosa with villous blunting



**Fig 18:** Photomicrograph of caecum revealing thickening of tunica muscularis with atrophy of the intestinal glands

### Conclusion

The Significant changes in the *E. coli* infected birds were seen in their haematological, serological and histopathological analyses. The pathomorphological alterations in the lymphoid organs including lymphoid depletion in the lymphatic follicles in spleen, bursa, thymus and caecal tonsils of infected broiler chickens were suggestive of immunosuppressive and immunomodulatory effects of *E. coli* in broiler chickens.

### Acknowledgement

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