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## Mortality pattern and pathological study of *E. coli* infection in broiler chickens

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### Abstract

The present study was carried out on broiler birds belonged to different farms and age groups to know the incidence, mortality pattern, gross and microscopic lesions of the *E. coli* infection. Detection of *E. coli* infection was done by cultural isolation, staining properties, and biochemical test. The farm wise mortality due to *E. coli* infection from thirty different flocks varied between 0.92-6.39 percent with an average 2.18 percent. Gross lesions of the nature of fibrin covering on the surface of liver and heart were diagnostic of the disease condition. Histopathologically liver, heart and lung showed multiple areas of focal necrosis, congestion, haemorrhage, oedema and fibrinous thickening along with mononuclear cells infiltration whereas, spleen showed similar lesions like heart, liver and lung with lymphocyte depletion. It is concluded that the colibacillosis is one of the most prevailing condition among broiler birds responsible for heavy economic loss to poultry industry.

**Keywords:** APEC, broiler, mortality pattern, pathological lesions

### Introduction

India is an agriculture based country where farmers mostly engage themselves in agriculture and animal husbandry practices. Poultry industry in India is considered to be the fastest growing among the agriculture based industries. The poultry industry in India as a whole is facing problems due to affection of infectious and non-infectious diseases. Among the infectious diseases avian colibacillosis is considered as one of the principal cause of morbidity and mortality in birds and associated with heavy economic losses to the poultry industry with its association with various disease conditions either as primary pathogen or as a secondary pathogen. It is characterized in its acute form by septicemia resulting in death and in its subacute form by pericarditis, air sacculitis and peri-hepatitis (Kuldeep *et al.*, 2013). Among various conditions caused by *Escherichia coli* organism, colisepticaemia is severe systemic form characterized by presence of *E. coli* in the blood and colonization in organs including the heart and spleen (Barnes *et al.*, 2003). However, Avian Pathogenic *E. coli* (APEC) often infects the respiratory system, resulting in the air sacculitis with airsacs thickened (Mellata, 2013) and caseous exudation in the respiratory tract and other systemic infections, including polyserositis, perihepatitis and pericarditis in the later stage of infection (Dho-Mulin, 1999). The pathogenic ability of *E. coli* strain is facilitated by broad range of virulence factors which are coded by virulence-associated genes (*iutA*, *iss*, *papC*, *iucD*, *tsh*, *irp-2*, *ompT*, *hlyF*, *iron*, *cva/cvi*, and *astA*). According to the genetic criteria, the pathogenicity of APEC strain is determined by presence of at least five virulence genes (De Carli *et al.*, 2015). In avian species, *E. coli* usually infects the respiratory tract, with bacteria passing through the mucosa, entering the blood stream, and causing the variety of diseases, including septicaemia, air sacculitis and pericarditis. Infection is generally enhanced by predisposing agents such as mycoplasma, viruses like IB, LPAI and environmental factors like heat, cold etc. (Gross, 1994). The present study was carried out to know the mortality pattern in different age group, gross and microscopic pathological lesions in case of *E. coli* infection in broiler chicken along with isolation, identification and biochemical characterization of *E. coli* organism.

### Materials and methods

The study was conducted on 30 different broiler farms (2-6 weeks of age) suspected of *E. coli*

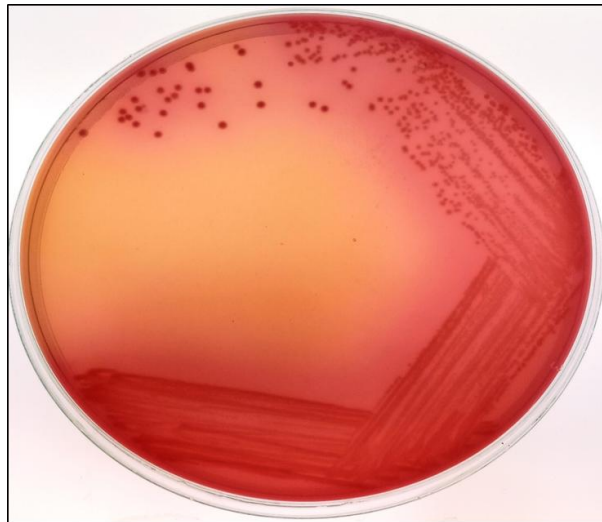
infection which were brought to the Departments of Veterinary Pathology for post-mortem examination during the period from August, 2018 to January, 2019. Pooled samples of heart, liver, lung and spleen from each flock showing gross lesions suggestive of *E. coli* infection were collected for microbiological analysis. A total of such thirty pooled samples were collected from 30 different farms. Individual pooled sample collected from each farm was thoroughly mixed and streaked on McConkey agar plates. The inoculated media plates were incubated aerobically at 37°C and inspected for growth after 24 hours of incubation. The pink colored colonies developed after 24 hours were selected from each inoculated plate and subcultured on Eosin Methylene Blue (EMB) Agar. After incubation, bacterial colonies were investigated and identification was done on the basis of staining, colony morphology, cultural and biochemical characters of pure isolates. This was carried out by using standard bacteriological and biochemical procedures as described by Carter and Cole (2012) and Barrow and Feltham (1993) [9, 5]. The overall incidence and mortality of colibacillosis was studied based on bacteriological examinations. To study the age wise prevalence of colibacillosis, total birds were categorized into five age groups viz. 2<sup>nd</sup> week (8-14 days), 3<sup>rd</sup> week (15-21 days), 4<sup>th</sup> week (22-28 days) and 5<sup>th</sup> week (29-32 days). During postmortem examination all organs of the dead birds were

examined thoroughly to detect gross lesions. During necropsy, organs including heart, liver, spleen and lung were collected in 10% neutral buffered formalin. Tissues of all organs were processed in tissue processor and then paraffin blocks were prepared. Sections were cut at 5-6 microns thickness and stained with Haematoxylin and Eosin (H & E).

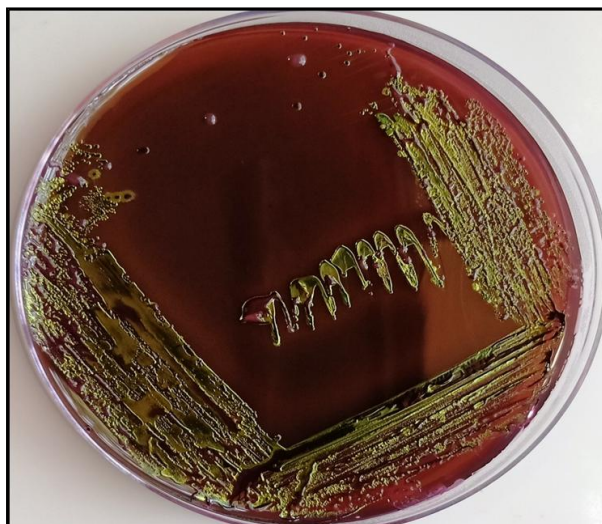
## Results and discussion

### Cultural Isolation and Identification of *E. coli*

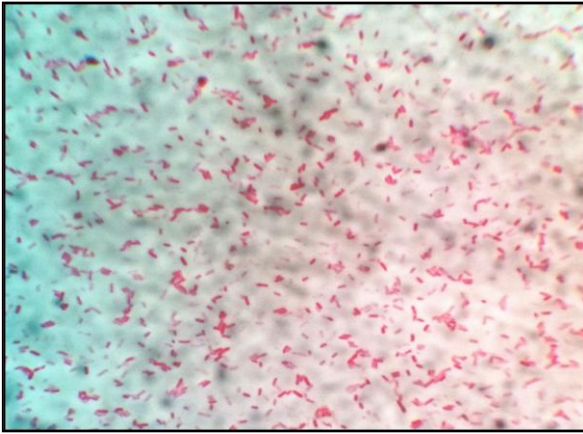
The pooled tissue samples like liver, heart, lung and spleen (pooled samples from each flocks) were streaked on MacConkey (MCA) agar and pink coloured colonies on MCA plates were considered to be lactose fermenting isolates (fig.1). Such pink colonies from each MCA plate were picked up and transferred to Eosin Methylene Blue agar (EMB) plate. Colonies with metallic greenish sheen on EMB agar plates were tentatively considered to be positive for *E. coli* (fig.2). A loopful colony was picked for Gram staining, which revealed pink coloured gram negative bacilli (fig.3). All the thirty isolates obtained were characterized by biochemical test i.e. indol production, Methyl Red (MR), Voges-Proskaur (VP) and citrate utilization test and the result were interpreted as per Bacteriological Analytical Manual (US FDA: BAM, 2002). All the isolates exhibited similar IMViC pattern of ++ -- (Fig.4).



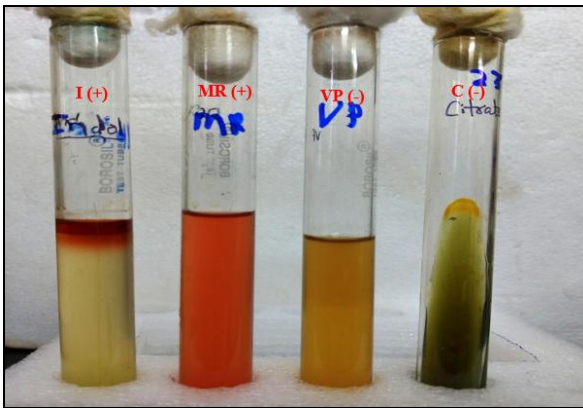
**Fig 1:** Lactose fermenting pink colour colonies of *E. coli* on MacConkey agar



**Fig 2:** Greenish metallic sheen produced by *E. coli* on EMB agar



**Fig 3:** Micrograph revealed pink coloured gram negative bacilli (*E. coli*)



**Fig 4:** Photograph showing result of IMViC test for *E. coli* (+++-)

### Farm Incidence and Mortality

Farm wise study of natural outbreaks of *E. coli* infection was conducted during the period of six months i.e. from August, 2018 to January 2019. The study comprised of the incidence of *E. coli* infection observed in 30 flocks of different broiler farms totalling the population of 1,26,400 birds of second, third, fourth and fifth week of age. The strength of affected flocks varied from 1500 to 10000 birds. The observations regarding flock wise, age wise and overall mortality due to *E. coli* infection were obtained by analysing the data collected from owner of the farm.

A total number of 2758 birds were reported to have died due to *E. coli* infection in thirty different flocks having total strength of 1,26,400 birds, with the overall mortality of 2.18 per cent. The mortality due to *E. coli* infection among these broiler flocks varied from minimum of 0.92 per cent to maximum of 6.39 per cent. However, in most of the flocks the mortality ranged between 1.00 to 4.00 per cent.

The available reports of earlier authors showed high variation in mortality pattern of broiler birds due to *E. coli* infection. Very high occurrence of mortality was reported in broiler chickens affected with colibacillosis and the rate was 87.5 per cent [14]. Similarly, *E. coli* was isolated from heart, blood and liver (86.6%) indicating systemic infection in broiler birds [6]. It was carried out on occurrence of infectious diseases in broilers chickens and observed 52.26 per cent mortality due to *E. coli* infection [2].

On the contrary, The prevalence of poultry diseases recorded 4.42 per cent incidence of colibacillosis in broiler birds [12]. Due to *E. coli* infection 6.56 per cent mortality also recorded in broilers chickens [13]. In research study 19.23 per cent

mortality was observed in broiler birds due to colibacillosis. Overall mortality and prevalence of colibacillosis was also reported 3.09 per cent mortality in the broiler flocks [8]. However, a very low level of prevalence rate i.e. 0.84 per cent was also recorded due to colibacillosis in broilers [3, 16].

### Age wise disease profile

Among different broiler flocks, majority of flocks (9 out of 30) were received for post mortem diagnosis of *E. coli* infection during third week of age followed by fourth and fifth (8/30 each) and second (5/30) week of age, appearing third week being the susceptible age for *E. coli* infection. The week wise mortality was in increasing order from second to fifth week of age when mortality due to *E. coli* infection was considered on the basis of gross total number of birds in the flock of representative age group. The week wise average mortality was 1.64, 1.89, 2.32 and 2.50 per cent during second, third, fourth and fifth week of age, respectively.

The age wise variation in an incidence of *E. coli* infection also have been reported by earlier authors. It observed that there was high mortality rate due to *E. coli* infection during fourth week followed by fifth and second week of age, respectively [8]. It was also noticed that colibacillosis was present in different age groups but maximum mortality was evident in birds of 3-4 weeks of age [6]. The total of 268 carcasses of birds were collected from organized and unorganized farms located in and around Guwahati and showed highest prevalence of colibacillosis in 3-6 weeks of age and the lowest in 9-12 weeks of age [7].

It was also investigated that the overall mortality and prevalence of colibacillosis among broilers of different age groups and they found highest mortality in the age group of second week followed by third week, first week, fourth week and fifth week of age respectively [3]. Similarly, higher mortality was recorded due to *E. coli* infection in 25-30 days and lower in 31-35 days old broiler birds [16].

Farm wise incidence of *E. coli* infection when viewed with the earlier reports, it reflected that the *E. coli* infection can cause mortality between 2 to 3 per cent among broiler chicks. It was also seen that in the field condition also the outbreaks were reported majorly during third, fourth and fifth weeks of age in broiler birds.

### Gross lesions

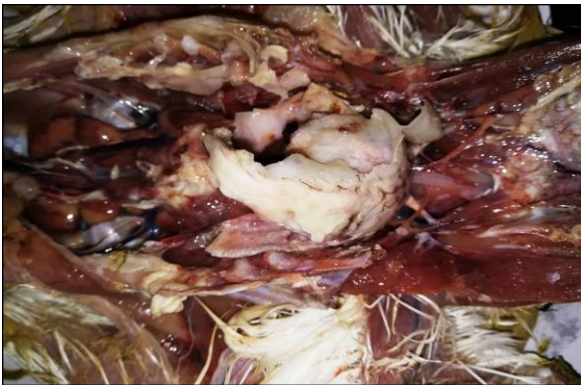
The gross lesions suggestive of *E. coli* infection were seen in all the affected birds. The main organs, which showed gross lesions were liver, heart, spleen, lung and air sacs. Most consistent and striking lesions were observed on the liver and heart in almost all the affected broiler birds. Typical white to yellowish fibrin layer over the surface of liver i.e. fibrinous perihepatitis and Fibrin layer over the surface of heart i.e. fibrinous pericarditis was observed in majority of the birds (Fig.5; Fig.6). In some birds the fibrin layer was thin and some had thicker layer over the organs. The fibrin covering over the heart was found to adhere with visceral surface of breast muscle in several cases (Fig.7). In some cases splenomegaly, hepatomegaly and congestion of heart, liver, lung, spleen and cloudy air sacs with deposition fibrinous mass were observed (Fig.8). Septicemic lesions with peritonitis, pericarditis and perihepatitis were also observed in almost all cases.

Similarly, lesions like fibrinous perihepatitis, fibrinous pericarditis and air sacculitis observed in the present study were similar to lesions observed by Nakamura *et al.* (1985),

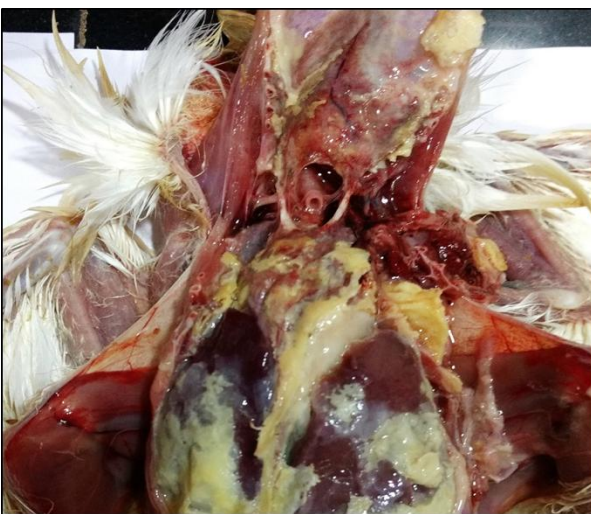
Pourbakhsh *et al.* (1997), Barnes and Gross (1997), Dhoulouin and Fairbrother (1999) during their study outbreaks of colibacillosis in poultry [18, 19, 4, 10]. Similar lesions were observed by Dutta *et al.* (2013) in pigeons affected with colibacillosis [11]. Gross lesions like congestion of heart, liver, lungs and spleen, accumulation of fibrinous exudates on pericardium, epicardium, hepatic surface, abdominal cavity, serosal surface of gastrointestinal tract including gizzard and mesentery and air sacs were observed in natural cases of avian colibacillosis [11].



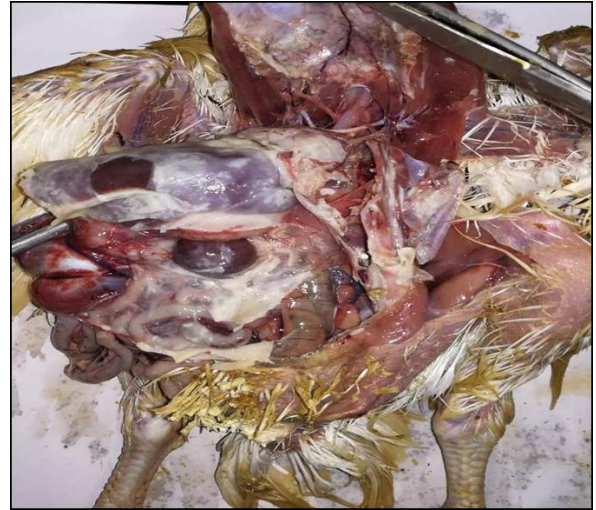
**Fig 5:** Gross photograph showing fibrinous covering over the liver (fibrinous perihepatitis) in broiler bird affected with *E. coli* infection



**Fig 6:** Gross photograph showing thick fibrinous covering over the pericardium (fibrinous pericarditis) in broiler bird affected with *E. coli* infection.



**Fig 7:** Gross photograph showing adherence of fibrin mass over pericardium to visceral surface of breast muscle to heart in broiler bird affected with *E. coli* infection.

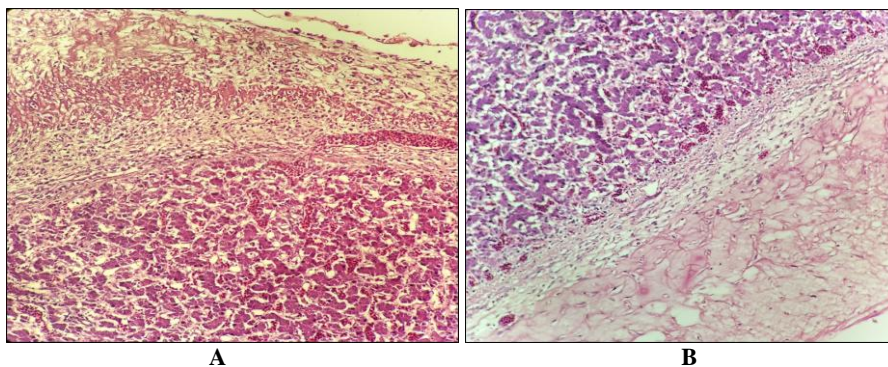


**Fig 8:** Gross photograph showing fibrin deposition along with fibrinous pericarditis and perihepatitis.

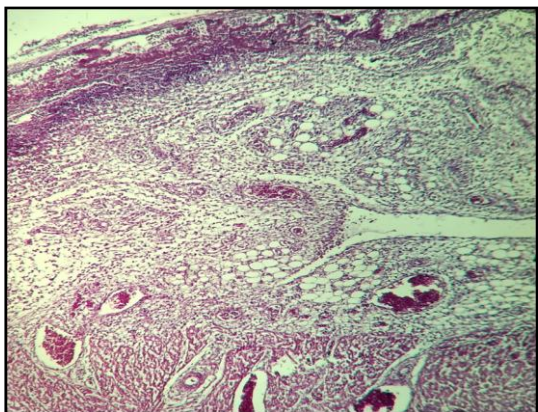
### Microscopic lesions

Liver were mild to moderate deposition of fibrin along with mononuclear cells infiltration on the outer surface of hepatic parenchyma i.e. fibrinous perihepatitis along with multiple areas of focal necrosis, mononuclear cells infiltration and mild to severe congestion in the hepatic parenchyma (Fig. 9-A & B). The lesions in the pericardium consisted of variable degree of fibrinous thickening i.e. fibrin covering over the surface along with mononuclear cells infiltration. Myocardium also revealed focal to diffuse mononuclear cells infiltration (Fig.10; Fig.11). Lungs showed the nature of congestion, haemorrhage, oedema, presence of fibrinous exudate and infiltration of mononuclear cells in the alveolar parenchyma. The lesions observed were variable severity (Fig.12; Fig.13). Microscopic lesions observed in the spleen were, multifocal necrotic areas along with mild to moderate congestion, presence of fibrinous exudate and lymphocyte depletion (Fig.14; Fig.15). Microscopically, it was noticed that there was large amount of fibrinous exudate on the surface of liver consisting of heterophils and lymphocyte, fibrin and degenerative changes in hepatocytes [5]. The severe congestion and haemorrhages, thick layer of fibrinous exudate with large number of heterophills and focal necrosis was observed over the surface of liver in pigeons affected with colibacillosism [11]. Jamoh *et al.* (2018) studied pathology of colibacillosis in broiler and layer birds and observed histopathological lesions like severe to moderate fibrino-perihepatitis, thickened hepatic capsule due to deposition of layers of fibrin with infiltration of mononuclear inflammatory cells, congestion and oedema [14]. In research studies on colibacillosis in broiler, layer or pigeon birds similar lesions of the nature of fibrinous thickening and congestion of pericardium along with mononuclear cells infiltration were reported time to time [19, 6, 11, 1, 15].

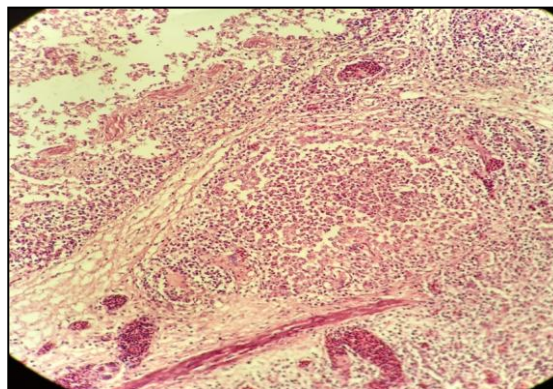
Similarly, Abalaka *et al.* (2017) observed histopathological lesions in the lungs like generalized perivascular and inter-septal oedema and haemorrhage during an outbreak of colibacillosis in broilers farms [1]. In experimental study on colibacillosis in turkey reported lesions like presence of fibrinous exudate and mononuclear cells infiltration [20]. Microscopic lesions of colisepticemia in chicken as multiple necrosis of the splenic lymphoid follicles accompanied with fibrinous exudation [18, 19]. Jan *et al.* (2018) observed degenerative and necrotic changes in the splenic cells with depletion of the nodules and disperse increase in lymphocyte population [15].



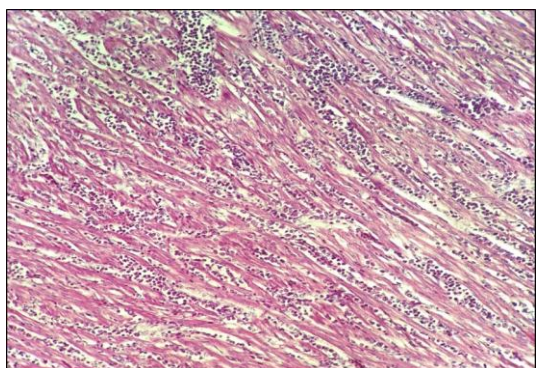
**Fig 9:** (A & B): Section of liver showing mild congestion, mild to moderate infiltration of mononuclear cells and fibrinous exudate on the surface of hepatic parenchyma (Fibrinous perihepatitis) in a broiler bird affected with *E. coli* infection (H & E stain, 240X)



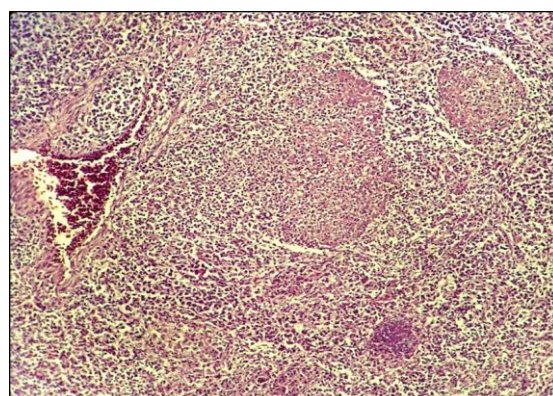
**Fig 10:** Section of heart showing pericardium with thick layer of fibrin, mild to moderate congestion and severe infiltration of inflammatory cells in broiler bird affected with *E. coli* infection (H & E stain, 120X)



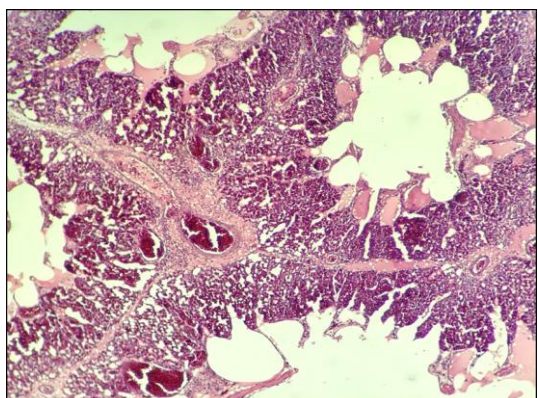
**Fig 13:** Section of lung showing mild congestion and mild to moderate infiltration of mononuclear cells in the alveolar parenchyma. (H & E stain, 240X)



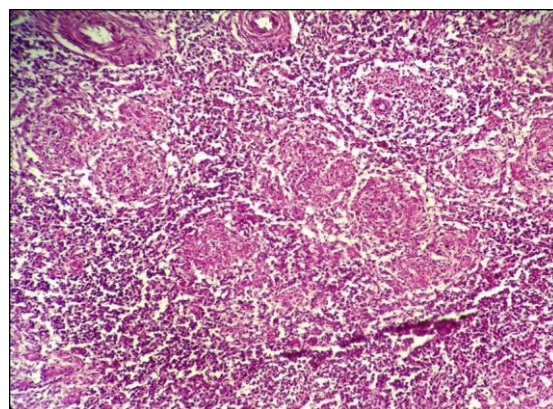
**Fig 11:** Section of heart showing mild to moderate mononuclear cells infiltration in the cardiac parenchyma. (H & E stain, 240X)



**Fig 14:** Section of spleen showing congestion of broiler birds affected with *E. coli* infection (H & E stain, 120X).



**Fig. 12:** Section of lung showing peri bronchial and peri vascular oedema and mild to moderate congestion in the alveolar parenchyma in a broiler bird affected with *E. coli* infection (H & E stain, 120X)



**Fig 15:** Section of spleen showing areas of necrosis with RE cell hyperplasia and depletion of lymphocytes in splenic follicles in a broiler bird affected with *E. coli* infection (H & E stain, 120X).

## Conclusion

On the basis of result obtained from the present study it is concluded that *E coli* infection is most commonly found bacterial disease in broiler birds affecting all age groups with varying mortality and showing gross pathological lesions i.e. fibrinous pericarditis/perihepatitis for disease identification.

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