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Pathological studies on avian pathogenic *Escherichia coli* infection in broilers

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Abstract
Colibacillosis is a complex syndrome characterized by multiple organ lesions like air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis or yolk sac infection. Pathological studies were undertaken on natural cases of avian colibacillosis to study the incidence and pathological lesions of *Escherichia coli* infection. Samples comprised of mortalities from various poultry farms operating in Srinagar, Pulwama and Ganderbal districts of Kashmir. Gross pathological changes included congestion in various organs, accumulation of fibrin on the liver and heart. Histopathologically, there was fibrinous pericarditis, myocarditis, fibrinous perihepatitis, hepatitis and fatty changes in hepatocytes, interstitial pneumonia, necrosis and depletion of lymphocytes in spleen. It is concluded that pathogenic *E. coli* in natural cases caused systemic lesions in chicks and also resulted in immunosuppression.

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

Introduction
Among poultry diseases, avian colibacillosis caused by *Escherichia coli* (*E. coli*) is considered as one of the principal causes of morbidity and mortality either as primary or as a secondary pathogen (Lutful Kabir, 2010) [15]. Sites of entry into the bloodstream are presumed to be the gas exchange regions of the lung and the air sacs, which are relatively vulnerable to bacterial invasion and colonization due to lack of resident macrophages (Mellata *et al.*, 2003) [16]. These infections occur in chickens of all age groups but broiler chickens within 4-6 weeks of age are more vulnerable and severely affected with considerable mortality (Leitner and Heller, 1992) [13]. Colibacillosis is a complex syndrome characterized by multiple organ lesions like air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis or yolk sac infection. In some instances, Avian Pathogenic *E. coli* (APEC) has been associated with peculiar diseases in specific avian species. In chickens, swollen head syndrome often results from synergistic infection of turkey rhinotracheitis virus and *E. coli* (Stehling *et al.*, 2003) [20]. Colibacillosis is one of the principal causes of morbidity and mortality in poultry worldwide. Especially it's most common form (Known as colisepticemia), an infection of the respiratory tract frequently followed by septicemia is responsible for high economic losses (Barnes and Gross, 1997 and Ewers *et al.*, 2003) [3, 5]. Death is the usual outcome of colisepticemia, but some birds may completely recover or recover with residual sequela as meningitis, Panopthalmitis (swollen eye), osteoarthritis, synovitis and coligranuloma (Hjarres's Disease: characterized by multiple granulomas in liver, caecum, duodenum and mesentery) (Barnes *et al.*, 2003) [4]. The present study was undertaken to elucidate pathological lesions in field cases of *E. coli* infected broiler chicks. The investigation on the field cases included postmortem examination, gross and histopathology.

Materials and Methods
Sampling
Samples comprised of mortalities from various poultry farms operating in Srinagar, Pulwama and Ganderbal districts and those which were brought to Division of Veterinary Pathology for post-mortem examination. 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. History of each suspected flock also included flock size, mortality and total number of birds per outbreak. Representative samples

(heart, liver, Intestine, lung and spleen) were collected in a sterile petridish for bacterial isolation and stored at 4ºC till inoculation in nutrient broth, followed by collection of tissue samples (heart, liver, lung, spleen, intestines, kidney and air sacs,) in 10% buffered formalin for histopathological examination.

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. 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 employing alcohol and acetone as dehydrating agent, benzene as clearing agent and paraffin wax of melting point 60 ˚C. The sections of 5µm thickness were cut and stained with Harris haematoxylin and eosin technique for routine examination and Alcian Blue PAS technique for demonstration of neutral and acid mucopolysaccharides (Luna, 1968) [14]. Demonstration of gram negative *Escherichia coli* in infected tissues was carried out by Taylor's method of staining. (Taylor, 1966) [22]

Results

Liver

Gross pathology: The liver of affected birds was covered by a thin fibrin layer in mild cases (Fig.1). In severe cases the fibrin layer was thick and covered whole liver giving characteristic "bread and butter" appearance (Fig. 2). Some of these livers were darkly discolored and revealed presence of focal areas of necrosis on the surface and were easily friable.

Histopathology: In early stages and mild cases of colibacillosis there was degeneration in the form of cellular swelling, individualization of hepatocytes along with distortion of hepatic cords (Fig. 3) Congestion of blood vessels and hemorrhages in liver was generally observed in all age groups. In some cases mild kupfer cell hyperplasia was also observed (Fig. 4). In severe cases, the capsule was excessively thickened characterized by presence of large amount of fibrin together with infiltration of numerous heterophils. Gram negative bacteria in the form of cocobacilli were observed when the tissue sections were stained by Taylor method.

Heart

Gross Pathology: Heart generally revealed congestion in most of the cases and it was usually covered with fibrinous layer which was thin in mild cases where as in severe outbreaks the heart was covered with thick layer of fibrin (Fig. 5).

Histopathology: In general there was thickening of pericardium with fibrinous exudate along with mononuclear cell infiltration (Fig. 6). In mild cases, the pericardium was slightly thickened with fibrinous exudate and heterophilic infiltration. In severe cases the pericardium was excessively thickened which also revealed eosinophilic necrotic areas containing heterophils in different stages of degeneration. In severe cases there was severe degeneration of myocardial muscle (myopathy) and in between muscle fibres, severe leucocytic infiltration predominantly heterophils was evident (Fig. 7).

Spleen

Gross pathology: Spleen in most of the cases generally was slightly enlarged and was congested (Fig. 8). In few cases however the spleen also revealed presence of necrotic foci scattered on its surface.

Histopathology: In severe outbreaks of colibacillosis especially during 3 - 4 weeks of age, there was severe congestion and hemorrhages in spleen together with thickening of blood vessels. Also depletion of lymphoid elements together with multiple focal areas of necrosis was evident (Fig. 9). Severe necrosis in the primary follicles of spleen resulted in the formation of secondary germinal centers in more severe cases (Fig.10). Depletion of lymphocytes at times was accompanied by reticular cell proliferation.

Lungs

Gross pathology: The lesions in lungs varied from mild congestion, oedema to consolidation. In severe cases lungs were severely consolidated and areas of consolidation were patchy to diffuse. The consolidation was usually unilateral in most of cases and in only few cases i.e. in advanced stages it was bilateral.

Histopathology: Lungs generally revealed congestion of interlobular septae and haemorrhages in the parabronchi. In few cases besides congestion and hemorrhages, there was mild infiltration of heterophils and mononuclear cells in the lumen of primary bronchioles and sometimes in tertiary bronchioles. In some cases there were acute bronchopneumonic changes in lungs characterized by presence of exudates in bronchioles and Para bronchi along with infiltration of leucocytes predominantly heterophils (Fig. 11).

Air sacs

Gross Pathology: Generally the air sacs were cloudy and in mild cases were covered with thin layer of fibrin (Fig. 12) and in severe cases the fibrin was thick. In advanced stages, air sacs revealed presence of caseous exudates on the surface. The lesions were more prominent on thoracic air sacs than the abdominal air sacs.

Histopathology: Histopathologically in all the cases, air sac membrane was diffusely thickened with leucocytic infiltration consisting predominantly of heterophils (Fig.13) The changes were frequently associated with presence of oedema and large amount of fibrinous exudate.

Kidneys

Gross Pathology: Generally in all cases of colibacillosis the kidneys were congested, swollen and edematous. Some of the cases also revealed presence of necrotic foci (Fig. 14) and pin point heamorrages on the surface of kidneys.

Histopathology: Kidneys in mild cases especially in birds of early age revealed varying degrees of congestion and haemorrhage in interstitial tissue accompanied by mild degeneration of tubular epithelium. In severe and advanced cases especially chicks of older age groups, besides congestion and haemorrhage, there was severe degeneration

of kidney tubules (Fig. 15) along with focal infiltration of leucocytes consisting of both mononuclear cells and heterophils.

Discussion

In past few years, both incidence and severity of colibacillosis have rapidly increased and current scenario alarms that it is likely to grasp its hold in future and thus impose a great threat to poultry industry (Altekurse *et al*., 2002) [1]. Gross pathological lesions were severe in liver which comprised of congestion, necrotic foci, deposition of fibrinous exudate on the surface of liver along with adhesions and rounding of edges, besides swelling of gall bladder and presence of fluid in abdomen. The results were in concurrence with the findings of Kumar *et al.* (2013) [11] who reported deposition of fibrinous exudate on liver surface besides other changes. Renu *et al.* (2012) [18] also reported thick fibrinous layer on all visceral organs in avian colibacillosis. Grossly heart generally revealed congestion, variable deposition of fibrinous layer on pericardium and adhesions of heart with chest cavity. Similar type of lesions have been described by Nakamura *et al.* (1985) [17] in a natural outbreak of colibacillosis in chicks, Gangane *et al.* (2006) in experimental colibacillosis, Renu *et al.* (2012) [18] and Kumar *et al.* (2013) [11]. Air sacs were generally cloudy and covered with a thin to thick layer of fibrin. Lesions were prominent in thoracic air-sacs than in abdominal air-sacs. The results are in concurrence with those of Sylevester *et al.* (2005) who also reported air sacculitis from clinical cases of different field outbreaks and Gangane *et al.* (2006) [6], who after inoculation of chicks with APEC intra-nasally reported congestion and cloudiness of air-sacs. Deposition of fibrinous mass on air sacs in natural cases of colibacillosis particularly in birds more than 3 weeks of age was also reported by Kumar *et al.* (2013) [11]. The fibrinous deposition on air-sacs could be attributed to severity of outbreak. Renu *et al.* (2012) [18] also reported cloudiness of air-sacs as one of the important pathological lesion in colibacillosis in chickens. Grossly lesions in the lungs varied from mild congestion to oedema and consolidation. Consolidation ranged from patchy to diffuse depending upon the severity of outbreak. Similar type of lesions have been observed by Gangane *et al.* (2006) [6] and Kumar *et al.* (2013) [11] which correspond to congestion, oedema and pneumonic foci. Also Tottori *et al.* (1997) [24] in an experimental study on colibacillosis reported congestion and pneumonic lesions in lungs. Grossly spleen revealed slight enlargement and variable degrees of congestion and presence of isolated necrotic foci in severe cases. The observations were in concurrence with the findings of Nakamura *et al.* (1985) [17] and Kumar *et al.* (2013) [11]. Generally lesions in kidney varied from congestion, oedema and in severe cases presence of pinpoint haemorrhages on its surface. The lesions were in concurrence with the findings of Baliar Singh *et al.* (1993) [2], who also reported congestion and oedema in kidneys in experimental colibacillosis. Similar changes were also observed by Gangane *et al.* (2006) [6] whereas Kumar *et al.* (2004) [12] reported that there were no appreciable changes in kidneys in colibacillosis affected chicken.

Histopathological changes observed in liver comprised of congestion, cellular swelling, and individualization of hepatocytes along with distortion of hepatic cords. These findings were in accordance with observations of Hooda *et al.* (2011) [9], Kumar *et al.* (2013) [11], Goyal *et al.* (2004) [12], Gangane *et al.* (2006) [6] and Hooda *et al.* (2011) [9]. Kumar *et al.* (2013) [11] reported deposition of large amounts of fibrinous exudate on liver, consisting of heterophills, lymphocytes, inflammatory cells, fibrin and degenerative changes in hepatocytes which were in accordance with observations in the present study. Hooda *et al.* (2011) [9] also reported dilatation of hepatic sinusoids, presence of RBC's in sinusoids, vacoulation and degeneration of hepatocytes, hyperplasia of Kupffer cells, congestion and haemorrhages. In heart the microscopic changes included thickening of pericardium with fibrinous exudates and cellular infiltration consisting of heterophils and mononuclear cells and necrosis of pericardium in severe cases. Myocardial changes include congestion, degeneration and haemorrhages along with varying degrees of leucocytic and mononuclear cell infiltrations in between myocardial fibres. These findings were in concurrence with observations of Kumar *et al.* (2013) [11], Renu *et al.* (2012) [18], Srinivasan *et al.* (2003) [19] and Jindal *et al.* (2003) [10]. Hepatic capsule is composed of connective tissue with poor cellular elements and blood vessels while as epicardium is rich in cells and blood capillaries. Therefore, adhesions between hepatic peritoneal sac and hepatic capsule might not be so strong and thus fibrinous layer accumulated on surface of liver were easily removable and not in case of heart, as it leads to strong adhesions with chest cavity and even with liver, which was comparable with current study. Microscopically lungs revealed varying degrees of congestion and haemorrhages in parabronchi. Mild heterophil infiltration in the air spaces was observed in mild cases and in severe outbreaks, focal areas of mononuclear cell infiltration, acute bronchopneumonic changes characterized by presence of exudates in bronchioles and parabronchi along with infiltration of leucocytes predominantly heterophils were evident. The described microscopic lesions were in concurrence to the findings of Tonu *et al.* (2011) [23] and Kumar *et al.* (2013) [11]. Changes in spleen included varying degrees of congestion, haemorrhages, focal areas of heterophil infiltration and depletion of lymphoid elements together with whitish multiple focal areas of necrosis along with reticulo-endothelial cell proliferation. The results were in concurrence with observations of Kumar *et al.* (2013) [11] and Hegazy *et al.* (2010) [8], who also reported depletion of lymphocytes in lymphoid organs in *E. coli* infection. The histopathological changes in kidneys consisted of congestion, haemorrhages, degeneration of tubular epithelium, focal areas of mononuclear cell infiltration and hypercellularity of glomeruli. The observations were in concurrence with those of Balair Singh *et al.* (1993) [2], who also observed kidneys revealing marked congestion and interstitial edema along with infiltration of mononuclear cells in colibacillosis. However, Kumar *et al.* (2004) [12], reported that *Escherichia coli* infected birds fed with ochratoxin A did not reveal appreciable changes in kidneys, bursa and thymus.

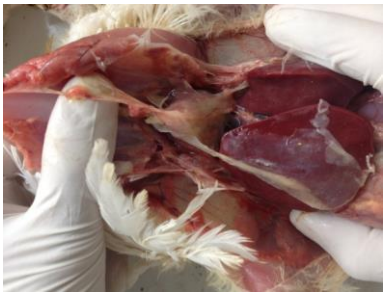

Fig 1: Broiler chicken affected with colibacillosis showing thin fibrin layer attached to liver.

Fig 2: Broiler chicken affected with severe colibacillosis showing thick fibrin layer attached to liver

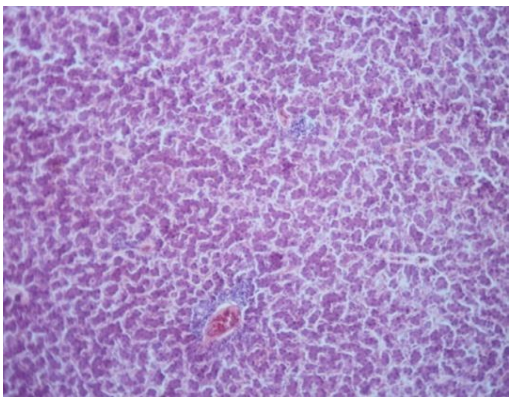

Fig 3: Section of liver from colibacillosis affected chicken revealing cellular swelling, individualization of hepatocytes along with distortion of hepatic cords. H.E. ×10

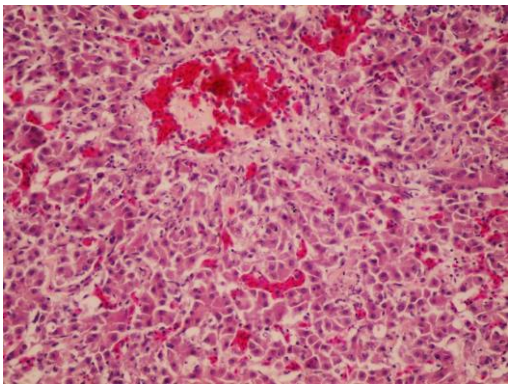

Fig 4: Section of liver from colibacillosis affected chicken revealing vascular congestion and mild kupfer cell hyperplasia. H.E. ×40

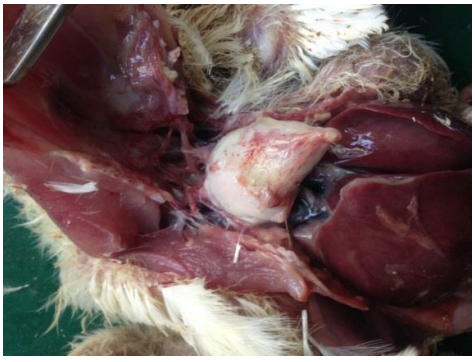

Fig 5: Broiler chicken affected with severe colibacillosis showing thick fibrin layer attached to heart

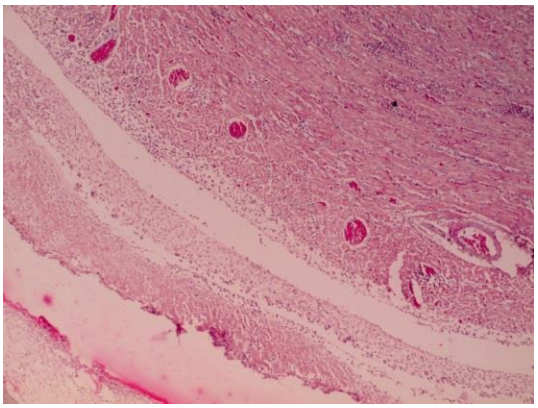

Fig 6: Section of heart from colibacillosis affected chicken revealing thickened pericardium along with infiltration by heterophils. H.E.×10

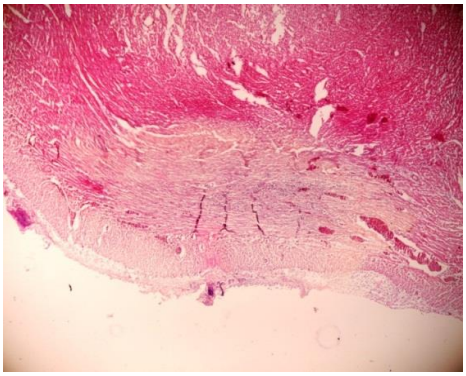

Fig 7: Section of heart from colibacillosis affected chicken revealing severe muscle degeneration (Zenker degeneration). H.E.×4

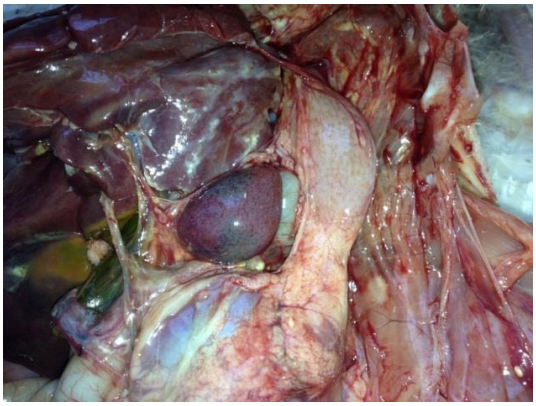

Fig 8: Broiler chicken affected with colibacillosis revealing enlargement and congestion of spleen.

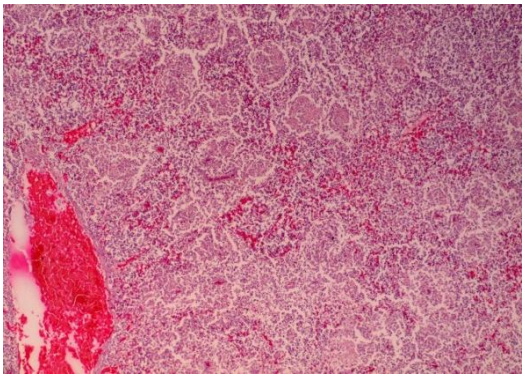

Fig 9: Section of spleen from colibacillosis affected chicken revealing multifocal necrosis along with depletion of lymphoid cells. H.E.×10

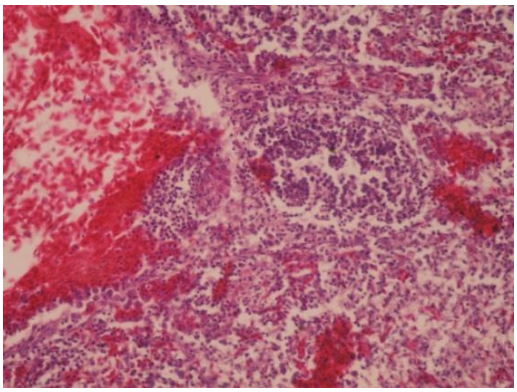

Fig 10: Section of spleen from colibacillosis affected chicken revealing formation of secondary germinal centers. H.E.×40

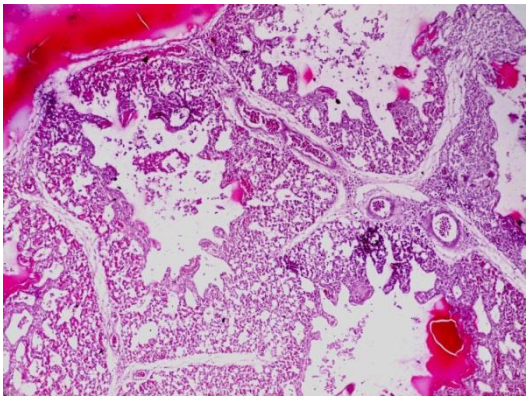

Fig 11: Section of Lung from colibacillosis affected chicken revealing presence of exudate in Para bronchi. H.E.×10

Fig 12: Chicken affected with colibacillosis showing fibrinous deposition on thoracic air sacs.

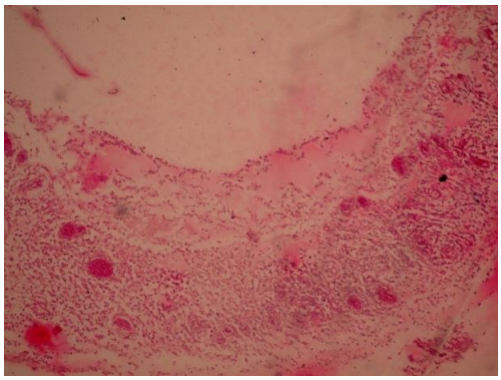

Fig 13: Section of air sac from colibacillosis affected chicken revealing necrosis, edema and infiltration. H.E. ×10

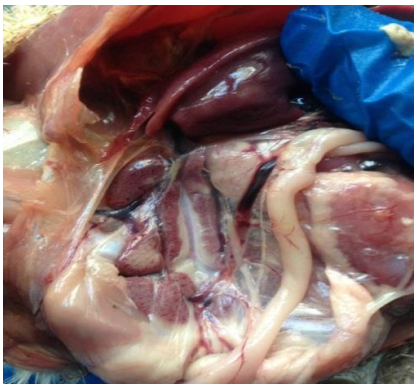

Fig 14: Colibacillosis affected broiler chicken revealing necrotic foci on kidneys.

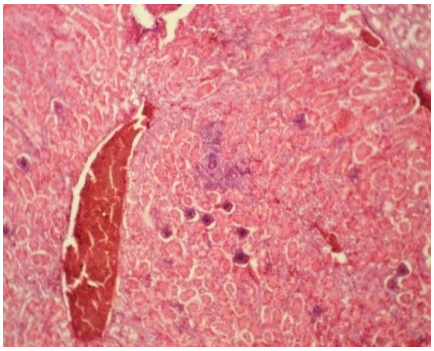

Fig 15: Section of kidney from colibacillosis affected chicken revealing severe degeneration of kidney tubules. H.E. ×40

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