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SHORT COMMUNICATION

Histopathological Findings and Apoptosis Caused by *E. coli* in Layer Birds

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ABSTRACT

In this study, our aim was to evaluate lesions seen in the tissues in terms of histopathology and to investigate the apoptotic cells seen in the tissues when *E. coli* outbreak occurred in a poultry farm. A total of 48 Lohmann White strains (53 weeks old) were submitted to the laboratory for necropsy. Microbiologic and histopathologic examinations were done on the samples taken from tissues. Apoptotic cells were determined in all of the tissues. The number of apoptotic cells increased as the tissue damage increased.

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INTRODUCTION

Escherichia coli (*E. coli*) bacteria are gram negative facultative anaerobic bacilli that are part of the normal intestinal microflora. Although most *E. coli* are non-pathogenic, some strains can establish themselves outside of the intestines and lead to disease. *E. coli* strains causing systemic disease in poultry are called avian pathogenic *E. coli* (APEC) (Dziva and Stevens, 2008). Colibacillosis is one of the main causes of morbidity, mortality and high economic losses in poultry industry (Tonu *et al*., 2011).

The most common lesions associated with colibacillosis are perihepatitis, airsacculitis and pericarditis, while other syndromes such as egg peritonitis, salpingitis, coligra-nuloma, omphlitis, cellulitis and osteomyelitis/arthritis may be encountered (Dho-Moulin and Fairbrother, 1999).

Apoptosis, or programmed cell death, is a physiological, genetically controlled, cellular response to external and internal stimuli whose purpose is to eliminate unwanted cells, including infected cells, while preventing damage to surrounding cells or tissue (Norbury and Hickson, 2001). There are many studies on bacteriological (Barnes *et al*., 2008) and histopathological (Tonu *et al*., 2011) changes in the *E coli* infections in poultry. However, a limited number of studies reported apoptosis caused by *E. coli*. This study was undertaken to histopathologically evaluate tissue damage caused by *E. coli* and determine relationship between this damage and apoptosis.

MATERIALS AND METHODS

E. coli outbreak occurred in a poultry farm in Erzurum. A total of 48 Lohmann White layers (53 weeks old) housed in cages (4 birds per cage) were submitted to the laboratory for necropsy. The birds were sacrificed for gut microbiology and histopathological examinations. Microbiological and histopathological examinations were performed on the samples taken from tissues.

Intestinal samples collected aseptically were streaked on 5% sheep blood agar EMB, McConkey's Agar and Selenite F agar. Microscopic examination was performed by Gram staining method. In samples of more than 10,000 *E. coli* colonies in caecum were identified microorganism (91%).

Tissue samples were fixed in 10% buffered formalin solution. After the routine histopathology process, microtome sections of 5 μm thick were prepared and stained with haematoxylin and eosin. Apoptotic cells were detected by terminal deoxynucleotidyl transferase-mediated dUTP nickend-labelling (TUNEL) stain using a commercial ready-to-use kit (In Situ Cell Death Detection Kit, POD, Roche, Mannheim, Germany). The procedure was carried out according to the manufacturer's instructions. The bacterial enumeration data were log-transformed prior to data analysis. The Proc Mean procedure was used to attain descriptive statistics. Data were expressed mean±SD.

RESULTS

Bacteriologic findings: In 48 samples, *E. coli* was the predominant bacterium (5.85±0.94) in small intestine, whereas *E. coli*, Candida spp. and Lactobacillus spp. were present at the number of 8.68±1.65, 8.18±0.13 and 8.05±0.56, respectively in caecum (data are log mean±SD).

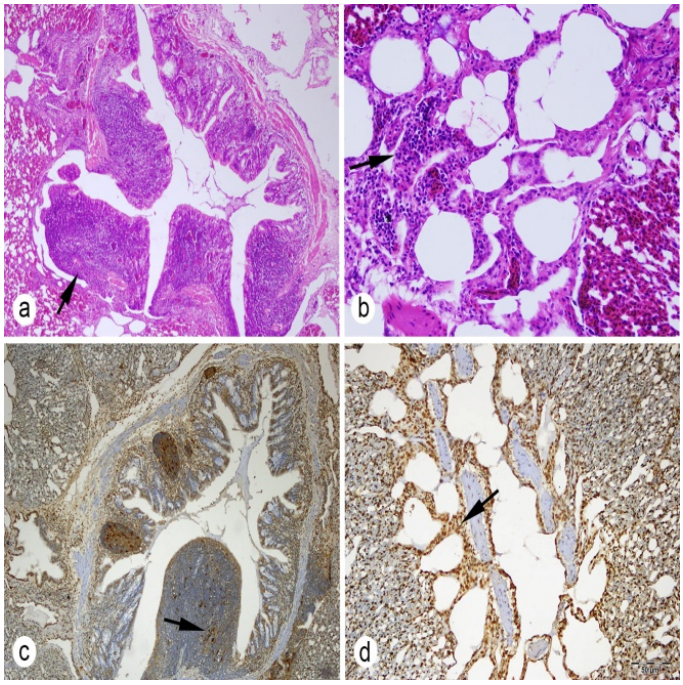

Fig. 1: Photomicrograph of lungs of broilers suffering from colibacillosis. a) Lymphoid hyperplasia and inflammation in bronchi (arrow), b) Inflammatory cells in the interlobular septa (arrow) and c-d) Apoptosis in bronchial epithelial cells (arrow). Stain: a & b: H&E; c & d: TUNEL; X20.

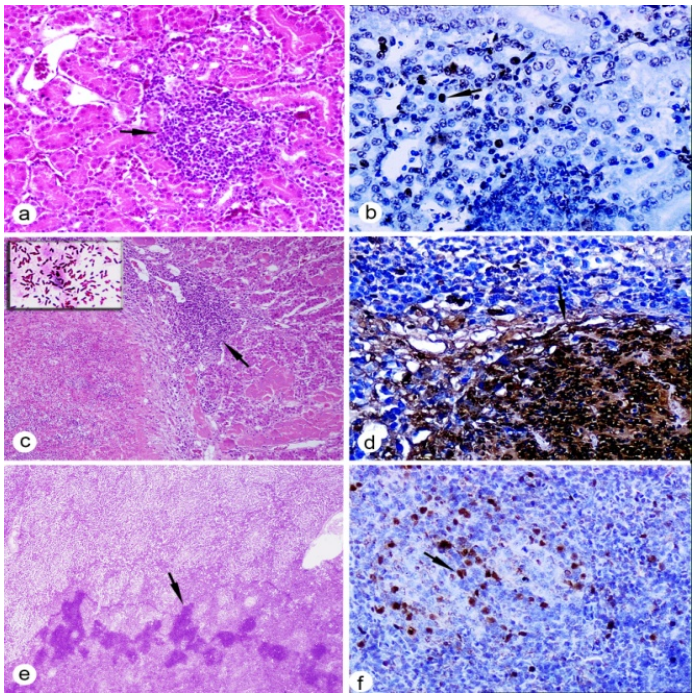

Fig. 2: Photomicrograph of various organs of broilers suffering from colibacillosis. a) Inflammatory cells in the interstitial area (arrow), b) Apoptosis in tubular epithelial cells and glomerulus (arrow), c) Necrosis and inflammatory cells in the liver (arrow). Above small plate: *E coli* (Gram stain, X 100), d) Apoptosis in the liver (arrow), e) Necrosis in the spleen (arrow) and f) Apoptosis in the spleen (arrow). Stain: a, c & e: H&E; b, d & f: TUNEL; X20.

Macroscopic findings: Clinically, weakness, in appetence, diarrhea were observed in the sick birds. Spleen, liver and kidney were markedly enlarged and congested. In some birds, foci varying in size and number, ranging in color from gray to yellow, were seen in the parenchyma of mentioned organs. The lungs were dark due to congestion. Some birds displayed thickening of the pleura and consolidated areas covered with yellowish fibrin in lungs.

Microscopic and immuno-histochemical findings: In the microscopic examination, the presence of necrosis or hyperplasia in the epithelium of the bronchi was seen. The presence of heterophils, lymphocytes, macrophages,

desquamated epithelial cells, cellular deposits composed of erythrocytes and mucus in the lumen was seen. Also, similar cells were seen in the interlobular septa and the pleura. Furthermore, lymphoid hyperplasia was noticed in the periphery of blood vessels and in secondary & tertiary bronchi (Fig.1a, b). In the liver, marked degenerative alterations of the hepatocytes were seen, while some were observed as necrotic. Furthermore, similar inflammatory cells were present in parenchyma (Fig. 2c). In the kidney, blood vessels were hyperemic, tubular epithelial cells were characterized by degenerative alterations and some were observed necrotic. In some birds, heterophils, lymphocytes and macrophages were seen in the interstitial area (Fig. 2a). The heart, blood vessels were hyperemic and heart muscle cells were degenerative. In a few cases, multifocal coagulation necrosis was present in the spleen (Fig. 2e). In addition, in only three birds, granulomatous foci were localized in the liver and lung.

Apoptotic cells were seen in all of the tissues. The number of apoptotic cells increased as the tissue damage increased. Apoptosis was observed in all tissues including bronchial epithelial cells in lungs (Fig.1c, d), hepatocytes in liver, (Fig. 2d), tubular epithelial cells and glomerulus in kidneys (Fig. 2b), myocytes in heart, white and red pulp in spleen (Fig. 2f), endothelial cells in vessels, and all inflammatory cells in varying proportions.

DISCUSSION

Avian colibacillosis is a contagious disease of birds caused by *E. coli* (Barnes *et al*., 2008; Dziva and Stevens, 2008), which is regarded as one of the main reasons of morbidity and mortality, connected with heavy economic losses to the poultry industry by its association with various disease conditions, either as primary pathogen or as a secondary pathogen (Tonu *et al*., 2011). Colibacillosis that can be localized or systemic can have a variety of symptoms (Dho-Moulin and Fairbrother, 1999). In the study, our findings were the same as the previous studies.

Coli-granuloma is a rare form of colibacillosis, which is characterized by granulomas in liver, caecum, duodenum and mesentery. Coli-granuloma was found in liver and lung in three of our cases. The histopathological changes observed in the present study had similarities to observations in earlier (Barnes *et al*., 2008).

Apoptosis occurs normally as a homeostatic mechanism to maintain cell populations in tissues during development and aging. Also, apoptosis occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or detrimental agents as well as a physiologically self-destruction of cell (Norbury and Hickson, 2001). It has been reported that apoptosis occurs in cases of infection such as, avian influenza virus (Mukherjee *et al*., 2012), hepatitis A virus (Sheng *et al*., 2014) and *Ornithobacterium rhinotracheale* infection **(**Terim Kapakin *et al*., 2013).

Apoptosis is often associated with bacterial infectious diseases in humans and animals, and caused substantial morbidity and mortality. Bacterial infections in particular play an important role in triggering apoptosis. Avian pathogenic *E. coli* from bacterial strains of *E. coli* that cause apoptosis, a process dependent on the activation of a cascade of caspases, present in the cytoplasm as zymogens (Bastiani *et al*., 2005), have selectivity for epithelial cell types (Gao *et al*., 2012).

There have been a limited number of studies regarding apoptosis in infections caused by *E. coli* in poultry. In those studies, it has been shown the presence of apoptosis in the heterophyl leucocytes (Bastiani *et al*., 2005), macrophages, epithelial cells of respiratory system (Horn *et al*., 2012) and intestine (Gao *et al*., 2012). However apoptotic changes occurring in other organs in *E. coli* infections in poultry have not been shown before.

In the present study, apoptosis shown in inflammatory cells and epithelial cells of the lung was consistent with the previous studies. In addition, apoptosis was determined in tubular epithelial cells and glomerulus in kidneys, myocytes in heart, white and red pulp in spleen, and endothelial cells in vessels.

Conclusion: *E. coli* was a powerful stimulus of apoptosis, as reflected by presence of a large number of the apoptotic cells in the inflammatory cells and lesion areas. Data suggest that prevention and treatment approaches should also cover cell or tissue maintenance or regenerations in order to compromise cell apoptosis and tissue damage in colibacillosis.

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