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

Kübra Asena Terim Kapakin¹, Samet Kapakin², Ali Kurt¹, Hatice Iskender³, Serdar Altun¹ and Demet Celebi⁴

¹Department of Pathology; ²Department of Anatomy, Faculty of Medicine, Ataturk University, 25240 Erzurum, Turkey
²Department of Nutrition and Dietetics, School of Nursing, Coruh University, Artvin, Turkey
³Department of Microbiology, Faculty of Veterinary Medicine, Ataturk University, 25240 Erzurum, Turkey

*Corresponding author: kbraterim@gmail.com

ARTICLE HISTORY (14-611)

| Received: | December 11, 2014 |
| Revised: | April 04, 2015 |
| Accepted: | June 17, 2015 |

Key words:
Apoptosis
Chicken
Escherichia coli

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.

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 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.

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).
Macroscopic findings: Clinically, weakness, inappetence, 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 multifocal coagulation necrosis was present in the spleen 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 rhinotratheale* 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 heterophil 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.

**Acknowledgement:** Thanks to Dr. A. Hayirli for helping in statistical analysis.

**REFERENCES**


Sheng XD, WP Zhang, QR Zhang, CQ Gu, XY Huang et al., 2014. Apoptosis induction in duck tissues during duck hepatitis A virus type I infection. Poult Sci, 93: 527-34.
