Journal of Animal Research: v.12 n.06, p. 979-985. December 2022

DOI: 10.30954/2277-940X.06.2022.22

Pathological and Apoptotic Studies of Oviduct in Layers with *E. coli* Salpingoperitonitis

M. Sasikala[1*], P. Srinivasan[2], V. Gowthaman[3], K. Sukumar[4] and A. Raja[5]

[1]Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, INDIA

[2]Department of Veterinary Pathology, Veterinary College and Research Institute, Salem, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, INDIA

[3]Poultry Disease Diagnosis and Surveillance Laboratory, Veterinary College and Research Institute Campus, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, INDIA

[4]Department of Veterinary Microbiology, Veterinary College and Research Institute, Salem, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, INDIA

[5]Education Cell, Veterinary College and Research Institute, Namakkal, INDIA

[*]Corresponding author: M Sasikala; E-mail: vetsasi.pathologist@gmail.com

Received: 26 Aug., 2022 **Revised:** 01 Nov., 2022 **Accepted:** 09 Nov., 2022

ABSTRACT

The reproductive system of female layer chicken was targeted by many infectious agents and causes oophoritis and salpingitis which results in drop in egg production. Bacterial agents cause egg drop by causing either salpingitis or salpingoperitonitis. The present study was undertaken to identify the pathological changes in oviduct in layer chicken with salpingoperitonitis. Heart blood, peritoneum and oviduct swabs were collected from affected chicken and screened for bacterial agents and representative tissue samples were collected in 10% NBF for histopathology and apoptosis. Out of 20 flocks, the *E.coli* alone was detected in 11 (55%) and remaining 9 flocks showed combined infection. The clinical signs of pale, shrunken comb and wattles, dullness and drop in egg production with more number of small eggs (5-7%) were noticed. The mortality rate of 1.2-2% could be recorded. Post mortem lesions recorded were thickened peritoneum with presence of milky fluid to caseous flakes and insipissiated yolk in the abdominal cavity. Oviduct showed marked thickening and lumen contained albuminous exudate to caseous flakes in the magnum and isthmus and thin shelled stained eggs covered with greyish yellow fibrin exudate in uterus and vagina. Histopathologically, all parts of oviduct showed degenerative and inflammatory changes. Apoptotic cells were seen in all parts of the oviduct. Apoptosis was more in magnum, uterus and vagina.

HIGHLIGHTS

- Gross and histopathologically, oviduct was severely damaged by *E.coli.*
- Oviduct showed large numbers of TUNEL positive apoptotic cells in the oviduct which indicates that *E.coli* act as powerful stimulant of apoptosis.

Keywords: *Escherichia coli*, Chicken, Salpingoperitonitis, Pathology, Apoptosis

Egg production is the major target for raising layer chicken flocks. The reproductive system of female layer chicken was targeted and damaged by many infectious agents, causes oophoritis and salpingitis which results in drop in egg production. *Salmonella sp., Mycoplasma gallisepticum, Escherichia coli and Hemophilus paragallinarum* are the bacterial agents which could cause

How to cite this article: Sasikala, M., Srinivasan, P., Gowthaman, V., Sukumar, K. and Raja, A. (2022). Pathological and Apoptotic Studies of Oviduct in Layers with *E. coli* Salpingoperitonitis. *J. Anim. Res.,* **12**(06): 979-985.

Source of Support: Non; **Conflict of Interest:** None

drop in egg production by causing either salpingitis or salpingoperitonitis (Elbayoumi *et al*., 2019; Hassan and Careem, 2020). Among the above, *E.coli* could be the more common etiology and it occurs as a single cause or combined infection. *E.coli* is a ubiquitous bacterium that present mostly as a commensal and a few virulent strains which are referred as avian pathogenic *E. coli* (APEC) and the disease caused by them is known as avian colibacillosis. Spread of *E. coli* infection from the reproductive tract to peritoneal cavity leads to salpingoperitonitis. Peritonitis can occur without salpingitis, but the combined lesions are more common (Nolan *et al*., 2013). Egg peritonitis also known as egg yolk peritonitis, yolk stroke, abdominal sepsis and abdominal septicemia is the common cause of sporadic death in layers or breeder hens, but in some flocks it became the main cause of death before or after reaching peak production and giving the appearance of contagious disease (Rosales, 2016). Salpingitis associated colibacillosis in a commercial layer flock was also recorded (Ozaki and Murase, 2009) and they observed thickened and edematous mucosa of the oviduct including vagina. Oviduct lumen contained masses of caseous exudates in the upper part and shelled egg covered with fibrinous exudates in the vagina. Egg peritonitis in commercial layer chicken is mainly caused by *E. coli* and responsible for 15.39% of reproductive abnormalities between 21 and 80 weeks of age (Srinivasan *et al*., 2013).

In response to internal or external stimuli, cells undergo a process called apoptosis, which is physiologically and genetically controlled and has the function of destroying undesired cells, without causing harm to neighboring cells or tissue (Norbuy and Hickson, 2001). Bacteriological (Barnes *et al*., 2008) and histopathological (Tonu *et al*., 2011) changes in various organs associated with *E.coli* infections of poultry were reported. However, a limited information was available on the pathomorphology and apoptosis in reproductive tract of layer chicken affected by *E.coli*. Hence the present study was undertaken to investigate the pathomorphological and apoptosis changes in various parts of oviduct in commercial layer chicken affected with *E.coli* associated salpingoperitonitis.

MATERIALS AND METHODS

Flock history

The study was conducted in commercial layer poultry farms located in Namakkal region of Tamil Nadu during the period from January 2021 to July 2022. A total of 1400 white leghorn layers, above 20 weeks of age from 70 flocks with the history of drop in egg production were examined for reproductive tract pathology. All the flocks were vaccinated as per standard vaccination schedule. The flocks were inspected during the period of increased percentile of drop in egg production and the information regarding age, strain of chicken, flock strength, method of rearing, vaccination history, source of feed and water, production performance including time of peak production, percentage of production, production drop and mortality were collected.

Necropsy, Histopathology and Apoptosis

The dead birds suspected for salpingoperitonitis were collected, surface disinfected and necropsies were performed as per standard procedure. Representative tissue samples were collected and fixed in 10% Neutral buffered formalin for histopathology and apoptosis. Samples were processed as per the standard techniques and 4 µm sections were prepared for histopathology and apoptosis. The sections were stained with Haematoxylin and Eosin for histopathological examination (Bancraft and Gamble, 2008). 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, ABCAM, HRP-DAB).

Etiological identification

Heart blood, peritoneum and oviduct swabs collected from 120 layer chicken from 20 flocks with salpingoperitonitis were screened for bacterial agents. Pooled tissue samples (spleen, caecal tonsil, oviduct, kidney, airsac, lungs and tracheal mucosal scrapings) were used for detection of viral agents like Ranikhet disease, Infectious bronchitis, Egg drop syndrome-76 and bacteria like M. gallisepticum and *M.synoviae* by PCR. Representative sterile swabs from heart, peritoneum and oviduct were inoculated in to nutrient broth and incubated at 37˚C for 24 hours following reinoculation on MacConkey agar and again incubated at 37˚C for 24 hours. The lactose fermenting pink colonies were re-inoculated on Eosin-Methylene Blue agar slants and incubated at 37˚C for 24 hours. Biochemical tests such

as IMViC reactions were employed for the confirmation of *Escherichia coli* (Murthy *et al*., 2008). To identify the source of *E.coli* infection materials such as cloacal swabs from apparently normal birds, pooled water samples from water tank and nipples were collected from the same flocks for bacteriological examination.

RESULTS AND DISCUSSION

Out of 20 flocks which showed the lesions of salpingoperitonitis, *E.coli* alone was detected in 11 (55%) flocks by cultural examination and remaining 9 flocks combined infection of *E.coli* with Newcastle disease virus (1/9), Mycoplasma Sp. (6/9) and Infectious bronchitis virus (2/9) was detected by Polymerase Chain using specific primers. The *E.coli* colonies appeared pink colored when grow on MacConkey agar plates and showed typical metallic sheen on EMB agar (Fig. 1). All the bacterial isolates in this study were positive for indole test, methyl red test and negative for Voges Proskauer reaction and citrate utilization test (Tonu *et al*., 2011). In the present study, *Escherichia coli* was isolated from the heart blood and oviduct swab collected from the salpingoperitonitis affected layer chicken with the history of drop in egg production. No bacterial growth was present in water samples collected from the water pipelines of the same poultry shed.

The clinical signs of pale, shrunken comb and wattles, dullness and drop in egg production (12-20%) with more number of small eggs (5-7%) were noticed in salpingoperitonitis affected layer flocks. The mortality rate of 1.2-2% could be recorded. These findings were inconsistent with the findings of Omer *et al*. (2010) who recorded the mortality of 6.8% in broilers and 1.9% in layers in an outbreak of colibacillosis. The incidence of salpingoperitonitis was recorded throughout the laying period but the incidence was more common during peak production from 25 to 60 weeks of age. It indicates that during peak production the birds are in high level of stress and most vulnerable for any infection. The similar findings were reported by Srinivasan *et al*. (2013) in commercial layer chicken with egg peritonitis. Post mortem examination revealed thickened peritoneum with the presence of milky fluid (Fig. 2) to caseous flakes and insipissiated yolk in the abdominal cavity (Kaikabo *et al*., 2007). Oviduct showed marked thickening and serosal vessels were congested (Fig. 3-a). On opening, oviduct mucosa including ampulla revealed thickening and edematous changes (Fig. 3-b). Oviduct lumen contained albuminous exudate to caseous flakes in the magnum and isthmus, thin shelled stained eggs covered with greyish yellow fibrin exudate in the uterus and vagina (Fig. 4-a) (Chaudri and Kariyawasam, 2014). Mucosal folds of the magnum showed severe congestion and isthmus mucosa showed moderate thickening. Uterus showed marked distension and on opening showed lumen contained varying sizes of thin shelled stained eggs and the mucosa

Fig. 1. *E. coli:* Pink colour colonies in MacConkey agar and metallic sheen in EMB agar

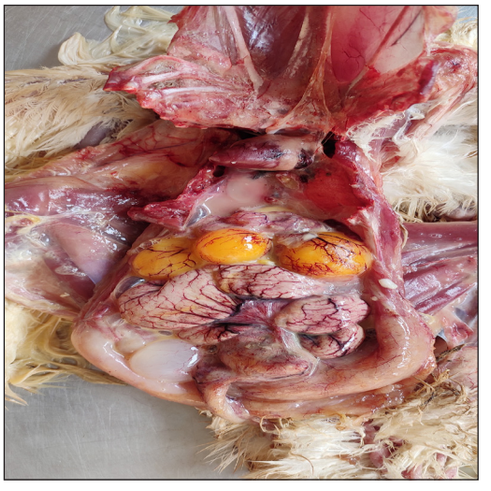
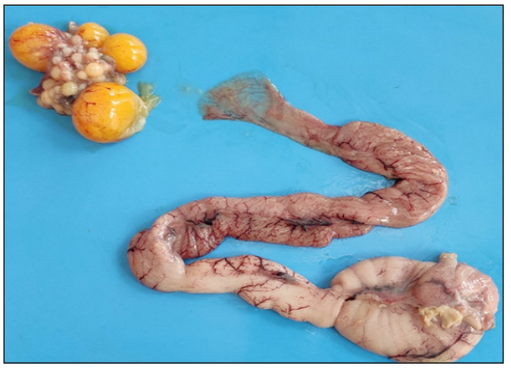


Fig. 2: Ruptured and flaccid ovarian follicles and accumulation of milky to caseous yolk material in the peritoneal cavity



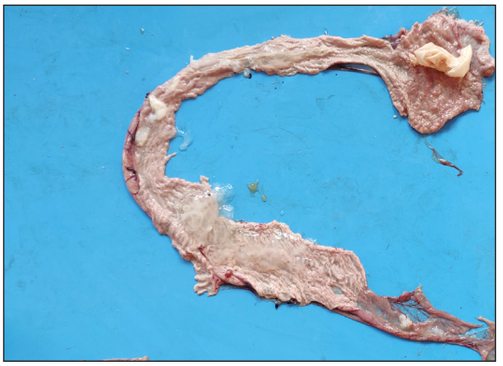


Fig. 3(a): Regressed ovarian follicles, congested and flaccid oviduct

Fig. 3 (b): Oviduct lumen showing albuminous to caseous exudate

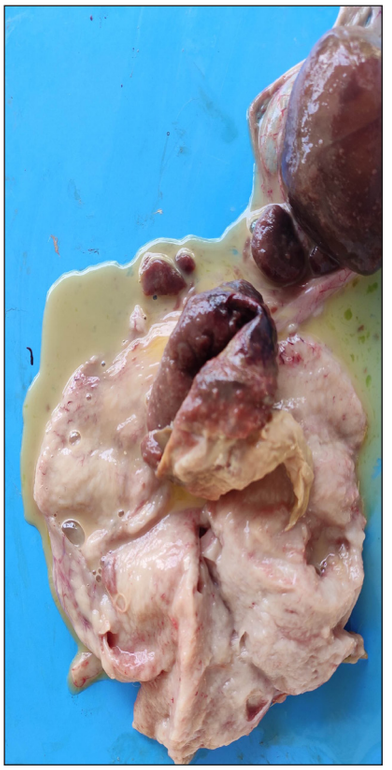


Fig. 4(a) Malformed eggs in uterus and vagina with severe inflammatory changes

Fig. 4(b) Severely thickened vaginal wall and lumen contained malformed eggs

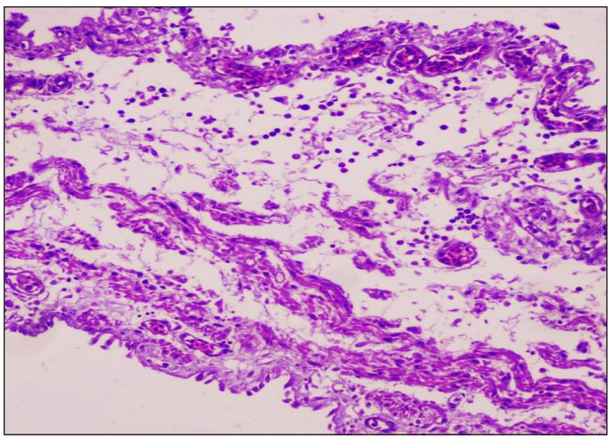


Fig. 5. Infundibulum: Congested, loss of surface epithelium, submucosal edema and mononuclear cell infiltration – H & E × 100

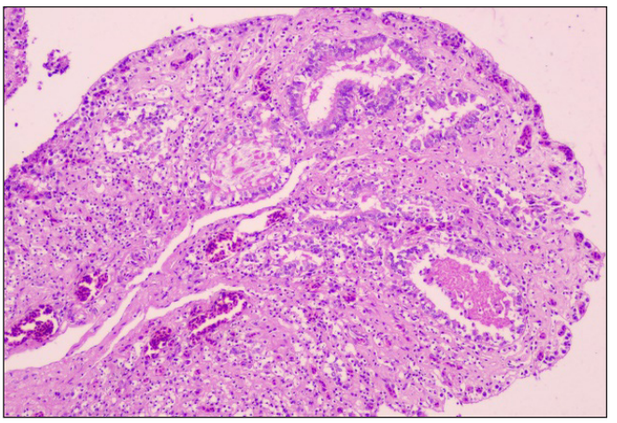


Fig. 6: Magnum: Congestion, complete loss of cilia, surface epithelium & tubular glands – H & E × 100

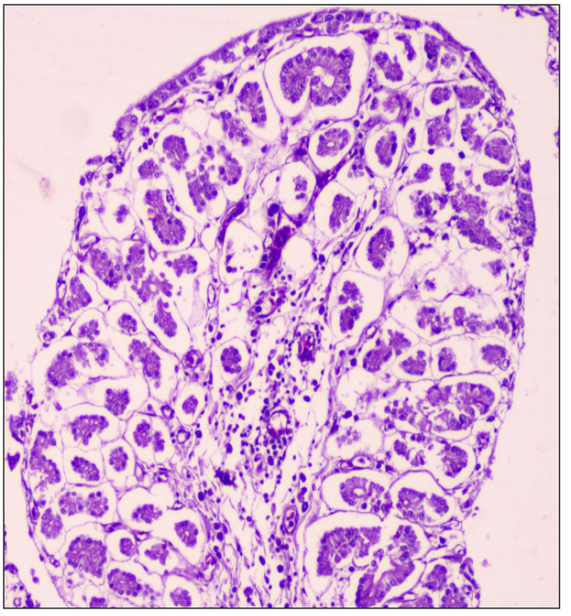


Fig. 7: Uterus: Loss of cilia & surface epithelium, tubular gland atrophy and mononuclear cell infiltration – H & E × 100

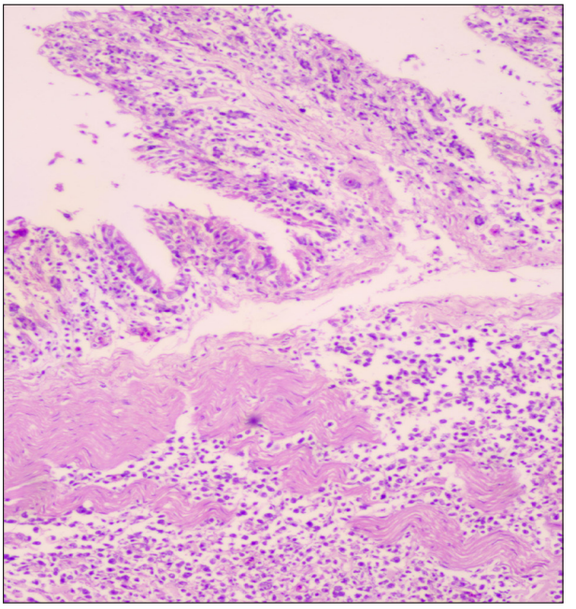


Fig. 8: Vagina: Atrophy of mucosal folds, severe mononuclear cell infiltration in the lamina propria and muscularis – H & E × 40

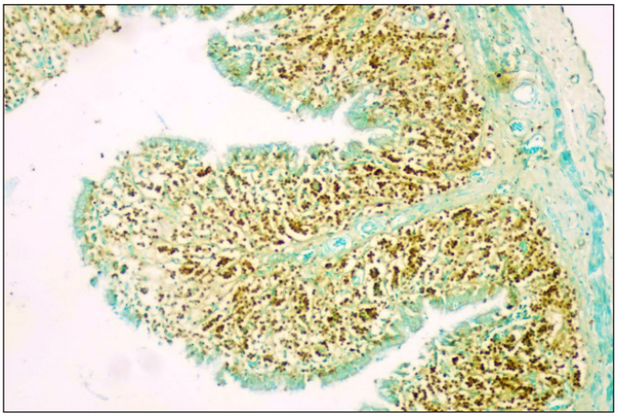


Fig. 9: Magnum showing numerous brown staining TUNEL positive (Apoptotic) cells in tubular glands and lamina propria – 40x

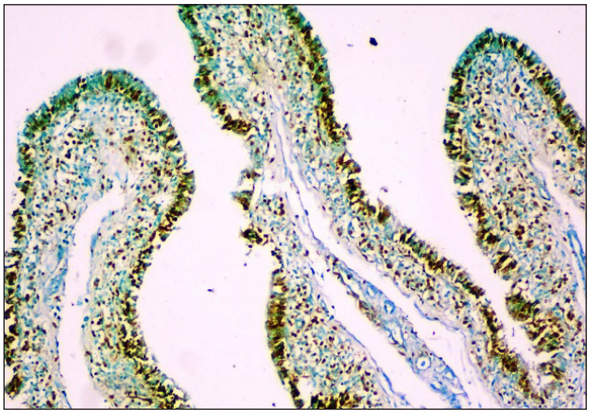


Fig. 10: Uterus showing positive brown staining TUNEL positive (Apoptotic) cells in surface epithelium and tubular glands – 40x

was severely thickened and revealed erosion. Vagina was greatly thickened and on opening muscularis showed severe thickening with malformed eggs (Fig. 4-b) (Ozaki and Murase, 2009). Ovarian follicles were congested, flaccid and some were atretic (Chaudri and Kariyawasam, 2014).

Histopathological examination of infundibulum showed severe thickening of wall due to submucosal edema. Mucosal epithelium showed loss of cilia, degeneration and lamina propria revealed mononuclear cell infiltration (Fig. 5). Magnum showed deciliation, loss of surface epithelium in many of the affected birds. Severe degeneration of tubular glands and few glands were regenerated and retain its secretion in the lumen. Intertubular connective tissue proliferation also seen (Fig. 6). Isthmus mucosa showed loss of cilia, degeneration and loss of surface

epithelium. Focal areas of mucosal folds showed marked infiltration of mononuclear cells and other areas also revealed mild mononuclear cell infiltration. Tubular glands showed degenerative changes and muscularis mucosa showed heterophilic infiltration. Uterus mucosal surface epithelium showed atrophy of secondary and tertiary folds, loss of cilia in surface epithelium, complete loss of tubular glands in the mucosal folds with infiltration of mononuclear cells. Lamina propria and muscularis mucosa revealed heterophilic infiltration (Fig. 7). Vaginal mucosal folds were short and showed severe congestion. The lamina propria and muscularis were thickened with marked mononuclear cell infiltration (Fig. 8). Along with cellular infiltration the muscularis showed edematous fluid accumulation. The histopathological findings were similar to the reports of Srinivasan *et al*. (2013).

Apoptotic cells were seen in all parts of the oviduct. The number of apoptotic cells was more in magnum, uterus and vagina (Fig. 9 & 10). The number of apoptotic cells in different parts of oviduct indicates the tissue damage induced by *E. coli*. The intensity of apoptotic cells was more in tubular glands of magnum, surface epithelium of uterus and muscularis region of vagina. The *Escherichia coli* invasion in to the tissue leads to cell injury which in turn shows a greater number of TUNEL positive cells. These findings were in accordance with the findings of Klumpp *et al*. (2001) who reported that *E. coli* infection associated with increased apoptosis in affected tissues. Avian pathogenic *E. coli* induce apoptosis by activating the caspases enzymes (Bastiani *et al*., 2005).

CONCLUSION

In the present study salpingoperitonitis in layer chicken was most commonly caused by *E. coli.* Out of 20 salpingoperitonitis affected flocks *E. coli* alone was detected in 11 (55%) and the remaining 9 flocks combined infection of *E. coli* with Newcastle disease virus (1/9), *Mycoplasma* Sp. (6/9) and Infectious bronchitis virus (2/9) were detected. Gross and histopathologically, oviduct was severely damaged by *E. coli.* Oviduct showed large numbers of TUNEL positive apoptotic cells in the magnum, uterus and vagina which indicates that *E. coli* induce severe damage in oviduct and act as powerful stimulant of apoptosis.

REFERENCES

Bancroft, J.D. and Gamble, M. 2008. Theory and Practice of Histological Techniques. 6th Edn, New York: Churchill Livingstone, Philadelphia.

Barnes, H.J., Nolan L.K. and Vaillancourt, J.F. 2008. Colibacillosis. 12th Ed, In Diseases of Poultry (Y.M. Saif *et al*.,); Blackwell Publishing, USA, pp. 691-732.

Bastiani, M., Vidotto, M.C. and Horn, F. 2005. An Avian pathogenic *Escherichia coli* isolates induces caspase 3/7 activation in J774 macrophages. *FEMS Microbiol. Lett.,* **253**: 133-140.

Chaudhari, A.A. and Kariyaswasam, S. 2014. An experimental infection model for *Escherichia coli* egg peritonitis in layer chickens. *Avian Dis*., **58**: 25-33.

Elbayoumi, K.M., Amer, M.M., Rabie, N. S. and Zaki, M.S. 2019. Conditions affecting egg production in chicken. *Report Opin.,* **11**(9): 8-17.

Hassan, M.S.H. and Careem, M.F.A. 2020. Avian viruses that impact table egg production. *Animals,* **10**: 1747.

Kaikabo, A.A., Musthapa, A., Yaroro, I.I. and Gashua, M.M. 2007. Occurrence of egg impaction and peritonitis in a flock of commercial laying hens in Damaturu Nigeria. *Niger. Vet. J.*, **28**(3): 56-58.

Kapakin, K.A.T., Kapakin, S., Kurt, A., Iskender, H., Altun, S. and Celebi, D. 2015. Histopathological findings and apoptosis caused by *E. coli* in layer birds. *Pak. Vet. J.,* **35**(4): 525-527.

Klumpp, D.J., Weiser, A.C. and Sengupta, S. 2001. Uropathogenic *Escherichia coli* potentiates type 1 pilus induced apoptosis by suppressing NF-kappa B. *Infect. Immunol.,* **69**: 6689-6695.

Murthy, T.R.G.K., Dorairajan,N., Balasubramaniam, G.A., Dinakaran, A.M. and Saravanabava, K. 2008. Pathogenic bacteria related to respiratory diseases in poultry with reference to *Ornithobacterium rhinotracheale* isolated in India. *Vet. Arh.,* **78**(2): 131-140.

Nolan, I.K., Barnes, H.J., Vaillancourt, J.P., Abdul-Aziz,T. and Logue, C.M. 2013. Colibacillosis. *In:* Swayne, D.E., Glisson, J.R, McDougald, L.R, Nolan, I.K, Suarez, D.L, Nair, V.L. (Eds.) Diseases of Poultry, 13th Edn. Iowa state press, Ames, IA, united states, pp. 751-806 (Chapter 18).

Norbury, C.J. and Hickson, I.D. 2001. Cellular responses to DNA damage. *Ann. Rev. Pharmacol Toxicol*., **41**: 367-401.

Omer, M.M., Abusalab, S.M, Gumaa, M.M, Omer, E.A, Jeddah, I.E, Hassan, A.M.A, Hussein, M.A. and Ahmed, A.M. 2010. Outbreak of Colibacillosis among broiler and layer flocks in intensive and semi-intensive poultry farms in Kassala state, Eastern Sudan. *Asian J. Poult. Sci.*, **4**(4): 173-181.

Ozaki, H. and Murase, T. 2009. Multiple routes of entry for Escherichia coli causing colibacillosis in commercial layer chicken. *J. Vet. Med. Sci.,* **71**(12): 1685-1689.

Rosales. A.G. 20163 Egg peritonitis in poultry. Merck and the Merck Veterinary Manual. Merck & Co., Inc., Kenilworth, NJ, USA.

Srinivasan, P., Balasubramaniam, G.A., Moorthy, T.R.G.K. and P. Balachandran, 2013. Bacteriological and pathological studies of egg peritonitis in commercial layer chicken in Namakkal area. *Asian. Pac. J. Trop. Biomed.*, **3**(12): 988-994.

Tonu, N.S., Sufian, M.A., Sarker, S., Karnal, M.M., Rahman, M.H. and Hossain, M.M. 2011. Pathological study on colibacillosis in chickens and detection of *E. coli* by PCR. *Bangl. J. Vet. Med.,* **9**(1): 17-25.