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Research Article

COLIBACILLOSIS ASSOCIATED SPONTANEOUSLY OCCURRING RESPIRATORY TRACT LESIONS IN BROILERS OF JAMMU

PRABJOT SINGH, SHILPA SOOD*, NAWAB NASHIRUDDULLAH, SHAFIQUR RAHMAN, PANKAJ GUPTA[1] and SATUTI SHARMA
Division of Veterinary Pathology, [1]Division of Veterinary Surgery and Radiology,
Faculty of Veterinary Science and Animal Husbandry,
Sher-e-Kashmir University of Agricultural Science and Technology of Jammu, R.S. Pura-181102, India

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ABSTRACT

Pathomorphology of respiratory tract infections in broiler chickens affected with *E. coli* was studied in 150 flocks from different localities in Jammu. 34.5% (19/55) organized farms and 31.57% (30/95) unorganized farms showed presence of a disease with respiratory involvement but *E. coli* was isolated from birds in 89.47% organized and 71.6% un organized farms surveyed which had outbreaks with involvement of respiratory tract. In nutshell, 32.6% of surveyed farms had respiratory tract infection of which 81.6% had an *E. coli* involvement. Among the confirmed colibacillosis cases, lungs were involved in 61.04% cases, 41.13% cases showed tracheal involvement, whereas 84.20% of cases showed air sac abnormalities. Apart from congestion, consolidation (19.8%), nodules (8.87%) and fibrin (23.76%) were visualized on lung surface. Microscopically, congestion, haemorrhage, edema, necrotizing pneumonia and serofibrinous pneumonia was seen in 75.18%, 36.52%, 79.43%, 10.28% and 13.12% lungs, respectively. 85.26% tracheal samples were congested, while haemorrhage, exudate and caseous plug was seen in 17.90%, 53.69% and 13.79% cases, respectively. Air sac thickening was mild, moderate and severe in 26.48%, 40.36% 33.16% cases, respectively. In conclusion, *E. coli* associated respiratory tract affections cause significant losses to farmers of broiler industry in Jammu and efforts must be made to mitigate this problem.

Keywords: Broiler, *E. coli*, Jammu, Pathomorphology

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Poultry industry has seen rapid development in India and population of poultry underwent an increase from 307 million (1992) to 851.81 million (2019) (20th Livestock Census, 2019). Currently, India ranks fifth in broiler-chicken production and total poultry population in Union territory of J &K is 8273709 (20th livestock census in J&K state). Morbidity and mortality as a result of different diseases affecting poultry cause huge monetary losses to the poultry farmers all across the world despite routine vaccination and other preventive measures. Diseases involving respiratory system comprise a significant proportion of pathological conditions affecting broiler population. The respiratory diseases have a complex etiology, often multiple pathogens are involved at a time which causes great economic impact due to losses in production and cost of treatment (Roussan *et al.*, 2008). Escherichia coli is an important pathogen affecting poultry birds causing colibacillosis, a generalised septicaemia infecting all age groups, which is characterized by air sacculitis, perihepatitis and pericarditis, but may involve respiratory tract and secondarily complicate outcomes in other respiratory infections such as infectious bronchitis and chronic respiratory disease. Inclement environment, age, immunity and presence of virulence factors in infecting strains can determine the susceptibility of host to *E. coli* infection. It was reported that in *E. coli* isolates belonging to O serogroup, presence of P fimbriae was associated with virulence characteristics such as hemolysis and invasiveness and hence caused a more severe disease outcome (Shankar *et al.*, 2010).

Since no study has been previously undertaken to determine occurrence of pathological conditions affecting respiratory tract of broilers of Jammu, so this study was planned to know gross and histopathological lesions affecting respiratory tract of broilers in Jammu occurring in association with *E. coli* infection.

MATERIALS AND METHODS

Study area: The study was conducted from December, 2018 to November, 2019 in and around Jammu which involved a total of 150 outbreaks from different poultry farms (55 organized and 95 unorganized) of Jammu. The study was undertaken in four seasons viz., monsoon (July to September), post monsoon (October to November), winter (December to February) and summer (March to June). Age and season wise occurrence and mortality pattern was calculated. Disease occurrence was determined as follows:

$$\text{Occurrence of disease} = \frac{\text{Total no. of morbidity + Total no. of mortality}}{\text{Total no. of birds in the flock}} \times 100$$

Mortality percentage was calculated as under:

$$\text{Mortality (\%)} = \frac{\text{Total no. of dead birds}}{\text{Total no. of sick birds in the flock}} \times 100$$

*Corresponding author: shilpasood@gmail.com

Necropsy examination: The routine necropsy protocol was followed. Any gross pathological lesion occurring in trachea, lungs, liver, kidney and air sacs of dead birds were recorded.

Bacteriological studies: Samples from trachea, lungs and air sacs (thoracic and abdominal) were collected under aseptic conditions from dead birds and were further processed for isolation of *E. coli*. Morphological, cultural and biochemical characteristics were used to identify the organisms. For confirming, *E. coli* organisms, swabs from lungs, trachea, air sacs were streaked on MacConkey agar and incubated at 37º C for 24 hrs. After incubating for 24 hours, Gram's staining was done on pure cultures. Pink coloured colonies from MLA (MacConkey's lactose agar) were also grown on eosin methylene blue agar (EMB). Cultures giving metallic sheen on EMB agar were also identified. Further characterization of *E. coli* was done on the basis of IMViC (Indole, Methyl Red, Voges Proskauer and Citrate) pattern (Quinn *et al.*, 1994).

Histopathology: For histopathological examination, representative tissue pieces from lungs, trachea, air sacs, kidney, and liver were preserved in 10% neutral buffer formalin and then processed as per the standard histopathological protocol. The paraffin embedded tissue sections were cut to a thickness of 4-5 micron with rotary type microtome and stained by routine haematoxylin and eosin (H&E) stain and mounted with DPX for observing the histomorphological changes.

Occurrence of gross and microscopic lesions was calculated as follows:

$$\text{Occurrence of lesions in an organ} = \frac{\text{Total no. of dead birds having a particular lesion in a particular organ}}{\text{Total no. of birds necropsied}} \times 100$$

RESULTS AND DISCUSSION

Occurrence of respiratory infections: Presently studied 150 flocks from different localities in Jammu included 55 organised and 95 unorganised poultry farms wherein 19 (34.5%) organized farms and 30 (31.57 %) unorganized farms showed involvement of respiratory tract, respectively. *E. coli* was isolated from 17 organized and 23 unorganized farms (Table 1). In nutshell, 49 (32.6%) of surveyed farms had respiratory tract infection in which 40 (81.6%) had an *E. coli* involvement.

Age wise prevalence of *E. coli* infection: Age wise occurrence of *E. coli* infection in the respiratory tract in the organized and unorganized farms is presented in Table 2. Percent of birds affected with *E. coli* which were under 10 days of age in organized/unorganized farms were 62 and 71%, respectively. Percent of affected birds in age group 11-40 days in organized and unorganized farms was 38 and 29 respectively. Overall, 30% and 31.35% of birds were affected with *E. coli* in organized and unorganized farms respectively. In another study undertaken in Hisar, Harayana, 10.97% mortality in chickens was reported due to occurrence of respiratory affections but highest mortality (30.88%) occurred due to the involvement of digestive system (Hooda *et al.*, 2011). Previous studies estimated occurrence of colibacillosis at 24.16% with a mortality of 8.33% in broilers in Jammu (Sanhga *et al.*, 2019). Ahmed *et al.* (2009) reported the occurrence of colibacillosis at Kapasia in Gazipur, Bangladesh to be 52.26% while Ahmed *et al.* (2012) found prevalence of colibacillosis was 18.61% in Poonch in Pakistan. Likewise, Hasan *et al.* (2010) recorded that occurrence of colibacillosis was 25.53% in Gazipur district.

E. coli infection was more prevalent among birds under 10 days of age in our study in both organised as well as unorganized farms. Janwari *et al.* (2019) did a study in Srinagar Ganderbal and Budgam on effect of season and age on mortality rate in broilers and found that mortality was highest in autumn, spring summer season followed by summer and winter, respectively also, the mortality was highest in young birds between age group 0-7 days and colibacillosis had the highest prevalence of 29.873% among all diseases. Matin *et al.* (2017) determined the occurrence of *E. coli* infection to be 1.0% and 0.5% in 25-30 days old and 31-35 days old broilers, respectively in greater Mymensingh district of Bangladesh with total occurrence of colibacillosis i.e., 0.84%. Borah *et al.* (2017) reported cases of colibacillosis throughout the year in Assam.

Season wise prevalence of *E. coli* infection: Season wise prevalence of *E. coli* infection is given in Table 3 and mortality pattern is shown in Table 4. Yearly mortality rate due to *E. coli* infection was found to be 11.01% with maximum mortality occurring in winter season. Abdeltawab *et al.* (2015) found that the cases of colibacillosis in broiler birds mostly occurred in both winter and summer season in Egypt. Likewise, Sultana *et al.* (2012) in Lahore Pakistan found 21.36% prevalence of colibacillosis among the broilers and the occurrence was more in winter even though cases were present throughout the year. Borah *et al.* (2017) found that among broilers in some districts of Assam namely Kamrup and Kamrup (Metro), incidence of colibacillosis was 11.11% which occurred throughout the year. Ibrahim *et al.* (2019) identified avian pathogenic *E. coli* from 53.4% of morbid chickens. Also, in turkeys, *E. coli* has been shown to be a significant problem as was demonstrated in a study by Kar *et al.* (2017), who confirmed 75%, 35% and 17.5% samples were affected, respectively by *E. coli*, *Salmonella* spp. or mixed *E. coli* plus *Salmonella*

Table 1. Prevalence (%) of *E. coli* infection in organized and unorganized farm

Parameter	Organised	Unorganised	Total
Total farm	55	95	150
Outbreak with Respiratory Tract infection	19 (34.5%)	30 (31.57%)	49 (32.6%)
Confirmed cases of *E. coli* infection in Respiratory Tract	17 (89.47%)	23 (71.6%)	40 (81.6%)

Table 2. Age wise (days) prevalence of *E. coli* infection in organized and unorganized farm

Farm type	Total farms	Total birds	Total affected *E. coli*		Affected (0-10 days)		Affected (11-40 days)	
			No.	%	No.	%	No.	%
Organised	17	34,000	10,200	30	6324	62	3876	38
Unorganised	23	7000	2195	31.35	1558	71	637	29

Table 3. Season wise prevalence of *E. coli* infection in poultry farm

Season	Total affected by *E. Coli* (12395)	% Affected by *E. Coli*
Monsoon (June - Sept)	3098	24.99
Postmonsoon (Oct- Nov)	1532	12.36
Winter (Dec- Feb)	6197	49.99
Summer (March - May)	1568	12.65

Table 4. Mortality pattern of affected birds in farm

Total affected	Total died	Mortality
12395	1365	11.01

infection. In this study, 24.99%, 12.36% and 49.99% cases due to *E. coli* infection were found in the monsoon, post monsoon and winter season, respectively. In summer season, total cases affected due to *E. coli* infection were 12.65%. Unlike our results, study by Islam *et al.* (2003) showed that avian diseases most often occurred in rainy season (56.36%) followed by summer season (28.11%) and winter (15.53%).

Organ affections in necropsied birds: A total number of 462 necropsies were conducted during the study period and lungs were found to be affected in 282 cases (61.04%), trachea was found to be affected in 190 (41.13%) cases and air sacs were affected in 389 necropsied birds (84.20%) cases. Ashraf *et al.* (2016) examined 451 organ samples including trachea and lungs revealed that 236 samples were positive for *E. coli* with an incidence rate of 52%.

Occurrence and severity of lung lesions: Upon opening rib cage, many birds had large quantities of edema fluid and flakes of fibrin which were adhering to the dorsal as well as undersurface of congested lungs. Overall, 172 lung samples recorded varying degrees of congestion. Out of the total congested, 88 (51.16%) were severely congested wherein the lungs appeared red and heavy due to edema (Fig. 1), 57 (33.14%) were moderately congested whereas 27 (15.70%) showed only mild degree of congestion. Other prominent lesions included consolidation, nodules and fibrin on the surface of lungs which were recorded in 56 (19.8%), 25 (8.87%) and 67 (23.76%) of cases, respectively.

Histologically, lung parenchyma had severely dilated and engorged capillaries, thickened septal walls on account of edema and congested capillaries. Exudate which was serofibrinous in character was present in the lumen of parabronchi and air vesicles. Around the congested vessels, perivascular accumulation of clear fluid consistent with edema was noticed. Often rupture of blood vessel wall leading to frank hemorrhage in the interstitium or alveoli was witnessed (Fig. 1b). Pneumonia was characterized by presence of congestion, edema along with infiltration of heterophils in parabronchi, atria and air capillaries. Focal as well as extensive areas of necrosis were infiltrated with viable as well degenerate heterophils. Bacterial colonies surrounded by heterophilic inflammation and karyorrhectic debris were also seen (Fig. 1c). Overall, microscopic examination of lung sections revealed presence of congestion, haemorrhage, edema, necrotizing pneumonia and serofibrinous pneumonia which were seen in 212 (75.18%), 103 (36.52%), 224 (79.43%), 29 (10.28%) and 37 (13.12%) cases, respectively.

Occurrence and severity of lesions in trachea: A total 190 trachea had one or more of the following gross alterations: congestion in 162 (85.26%), hemorrhage in 34 (17.90%), exudation in 102 (53.69 %) and caseous plug was seen in 26 (13.68%) of the examined trachea. Grossly, tracheal mucosa frequently showed diffuse reddening with slimy mucinous exudate overlaying a congested mucosa while in others the overlaying serofibrinous exudate appeared blood tinged. Moreover, in other cases severely congested trachea had mucopurulent exudate in its lumen which further became thick and caseous (Fig. 1d). In more prolonged cases, thick fibrino-necrotic plaques could also be appreciated on mucosal surface. Histologically, lamina propria in thickened tracheal epithelium was expanded due to severely engorged capillaries and heterophilic infiltrate (Fig. 1e). In some cases, the severely degenerated mucosa was expanded by edema and inflammation and was also ulcerated at places (Fig. 1f). Other changes included severe necrosis and edema of tracheal mucosa, denudation of tracheal epithelium, necrosis of tracheal epithelium, goblet cell hyperplasia, and severe serosal congestion. Depending on the predominant inflammatory constituent, tracheitis was categorized as purulent, 61 cases (32.11%); catarrhal in 52 cases (27.37%); haemorrhagic in 51 (26.84%) and serofibrinous in 26 cases (13.69 %). Chaudhari *et al.* (2017), also did a study to associate *E. coli* infection with stress on respiratory system in broilers and isolated *E. coli*

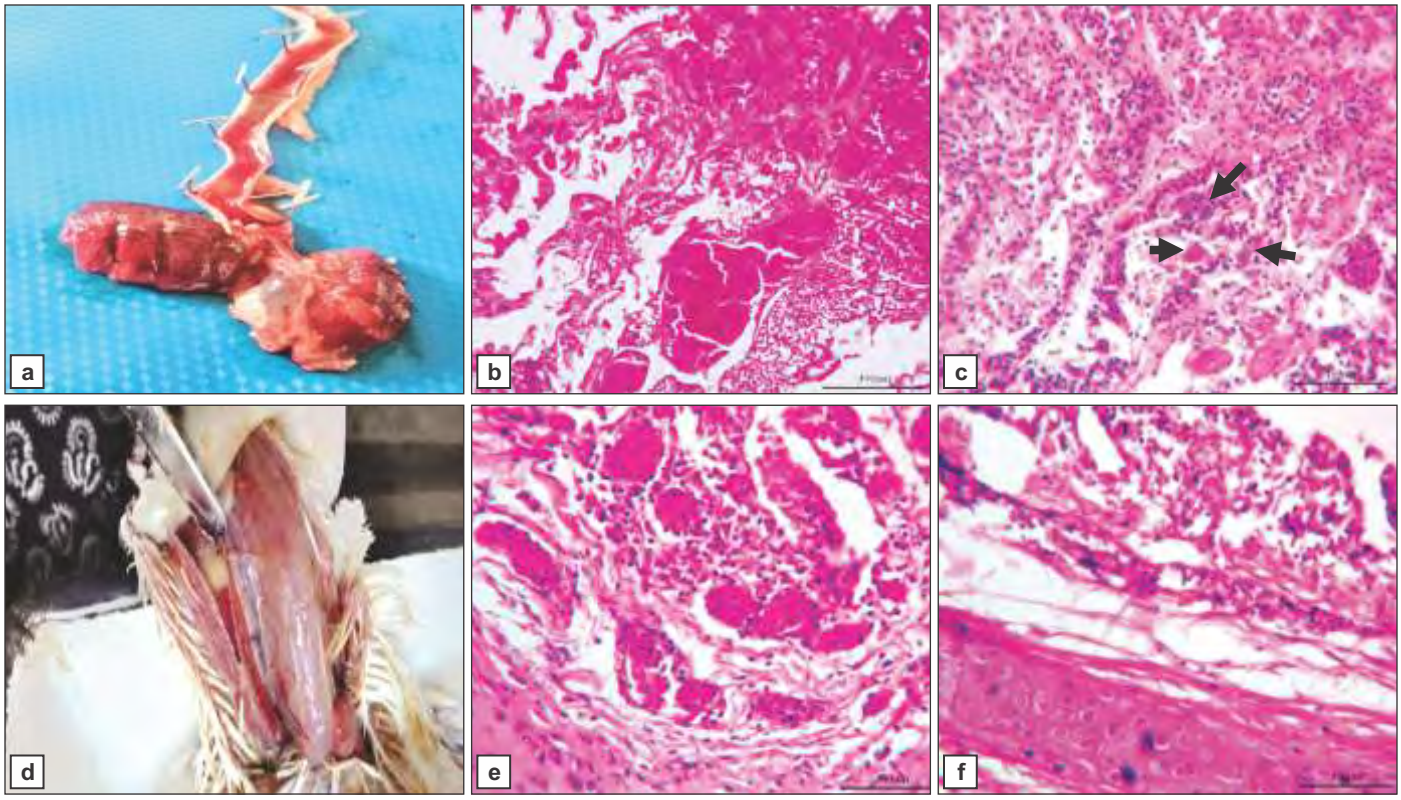

Fig. 1. **(a)** Congested lungs and trachea, **(b)** Lung showing congestion, haemorrhage, perivascular edema and inflammatory cells (H & E 100X), **(c)** Bacterial colonies (arrows) surrounded by fibrinosuppurative exudate (H&E 400X), **(d)** Trachea cut open to reveal severely congested tracheal mucosa with purulent exudate, **(e)** Severely congested capillaries in tracheal mucosa with edema and heterophilic infiltration (H&E 400X), **(f)** Necrosis of tracheal mucosa and infiltration of inflammatory cells in tracheal mucosa (H&E 400X)

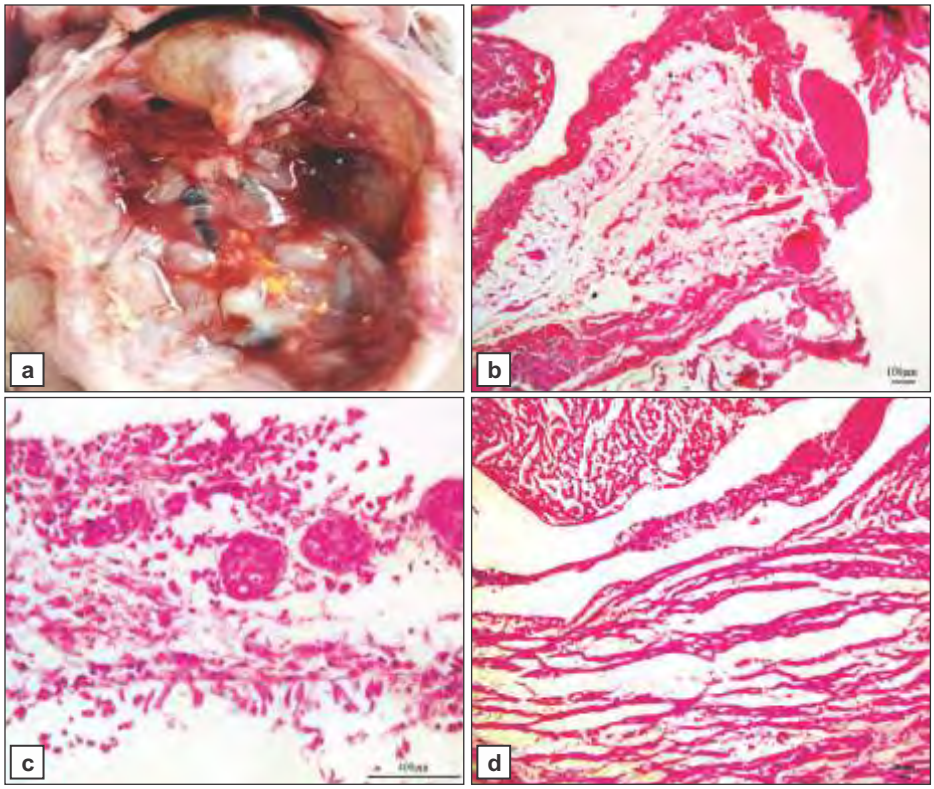

Fig. 2. **(a)** Fibrinous pericarditis and air sacculitis, **(b)** Severely thickened air sacs due to edema, congestion and fibrin (H &E 100X), **(c)** High power view of affected air sacs with congestion, fibrin and heterophilic infiltration (H&E 400X), **(d)** Thick fibrin layer in pericardium with inflammatory cells (H &E 40X)

from all the samples processed from birds with a bronchial plug.

Occurrence and severity of gross and microscopic lesions in air sacs: Gross examination revealed a variety of lesions in air sacs. A total of 389 air sacs were thickened. The air sacs appeared wet or slightly cloudy in less severe cases whereas in severe cases a thick sheet of fibrin covered the air sac surface (Fig. 2a). The thickening was mild in 103 cases (26.48%) whereas 157 air sacs (40.36%) were moderately thickened and 129 (33.16%) were severely thickened. Histopathologically, apart from the presence of serofibrinous exudate and congested capillaries, the air sacs also had heterophilic infiltration and fibrillar necrotic debris (Fig. 2b, 2c).

Prominent characteristic lesions in other organs included fibrinous pericarditis where a frosting of fibrin was appreciated over visceral organs notably heart (Fig. 2a) which upon

microscopic examination appeared as multiple layers of fibrinous exudate expanding pericardial sac (Fig. 2d). In almost all cases of colibacillosis, edema and congestion of kidney and fibrinous perihepatitis was present. Sangha *et al.* (2021) studied pathomorpholgical changes in cases of colibacillosis with emphasis on gastrointestinal tract of broilers and reported presence of hepatic necrosis with perihepatitis, proventriculitis and enteritis apart from pericarditis and air sacculitis in the affected birds. Randomly distributed multifocal necrotic foci on the liver surface, were also visualized. Surjagade *et al.* (2020) did an investigation to estimate prevalence and study the pathomorphological manifestations of colibacillosis from different parts of India in which an average mortality of 2.18% ranging between 0.92-6.39% was observed and fibrinous serositis with necrosis, edema, congestion and hemorrhage along with infiltration of mononuclear cells in cardiac, hepatic and lung parenchyma was seen. Likewise, Shah *et al.* (2019), undertook pathology based studies on naturally occurring cases of colibacillosis in Srinagar, Pulwama and Ganderbal districts in Kashmir and found, congestion, fibrinous perihepatitis, pericarditis and air sacculitis with fibrinoheterophilic infiltrates were seen in pericardium and Glisson's capsule along with fatty changes, necrosis and hepatitis, interstitial pneumonia and lymphoid depletion was observed. Also, fibrinous perihepatitis, fibrinous pericarditis and air sacculitis due to *E. coli* infection were also reported by Ali and Ali (2015). Itoo *et al.* (2014) studied respiratory tract abnormalities in broilers in Kashmir and reported that trachea, lungs and air sacs were affected in 46.79%, 55.43% and 44.79% of the necropsied birds, respectively. They also reported congestion, exudates, haemorrhage and caseous plugs in larynx and trachea, congestion, consolidation and nodules in lungs alongside cloudiness with thickening of air sacs due to fibrinous, caseous and urate deposits. Likewise, Rua *et al.* (2014) determined an overall occurrence rate of *E. coli* to be 37.5% and also recorded fibrinous pericarditis, fibrinous perihepatitis and air saculitis from the affected birds. Similarly, Thapa and Chapagain, (2020), performed an investigation on suspected outbreak of colibacillosis in layers and broilers in Chitwan, Nepal and identified airsaculitis, pericarditis perihepatitis and egg peritonitis as the most common manifestations of *E. coli* infection. Future studies must be carried out to further determine genetic lineage of *E. coli* associated with respiratory infections in broilers and role of other respiratory pathogens in the exacerbating lesion severity. Efforts must to made to reduce respiratory stress to birds, improve managemental practices and educate farmers to reduce economic losses attributed to *E. coli* infection in broilers.

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