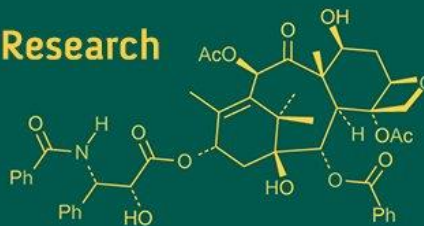
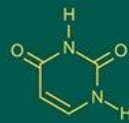


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(6): 1308-1317
www.biochemjournal.com
 Received: 17-03-2026
 Accepted: 20-04-2026

Savita Sahu
 Department of Veterinary
 Pathology, Dau Shri Vasudev
 Chandrakar Kamdhenu
 Vishwavidyalaya, Anjora,
 Durg, Chhattisgarh, India

Dhananjay Kumar Jolhe
 Department of Veterinary
 Pathology, Dau Shri Vasudev
 Chandrakar Kamdhenu
 Vishwavidyalaya, Anjora,
 Durg, Chhattisgarh, India

Ratan Chandra Ghosh
 Department of Veterinary
 Pathology, Dau Shri Vasudev
 Chandrakar Kamdhenu
 Vishwavidyalaya, Anjora,
 Durg, Chhattisgarh, India

Vivek Kumar
 Department of Veterinary
 Pathology, Dau Shri Vasudev
 Chandrakar Kamdhenu
 Vishwavidyalaya, Anjora,
 Durg, Chhattisgarh, India

Kamlesh Kumar
 MVSc, Department of
 Veterinary Microbiology,
 College of Veterinary and
 animal sciences, Anjora, Durg,
 Chhattisgarh

Corresponding Author:
Vivek Kumar
 Department of Veterinary
 Pathology, Dau Shri Vasudev
 Chandrakar Kamdhenu
 Vishwavidyalaya, Anjora,
 Durg, Chhattisgarh, India

Prevalence, pathology and molecular characterization of *Escherichia coli* in chickens

Savita Sahu, Dhananjay Kumar Jolhe, Ratan Chandra Ghosh, Vivek Kumar and Kamlesh Kumar

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6p.8898>

Abstract

This study investigated the prevalence, pathology, and molecular characterization of avian Colibacillosis in Chhattisgarh from November 2021 to July 2022. Examining 250 birds from 20 poultry farms, researchers identified 32 *E. coli* isolates, reflecting an 18.8% prevalence rate. The highest prevalence occurred in Durg (25%) and the lowest in Surajpur (12%). Recorded conditions included Colisepticaemia (59.57%), Omphalitis (31.91%), and Egg Peritonitis (8.51%), with pathology showing systemic inflammation, necrosis, and congestion. *E. coli* was confirmed via cultural isolation, biochemical tests, and PCR detection of the *tsh* gene. All isolates were Gram-negative, exhibited standard biochemical profiles (Indole+, MR+, Catalase+, VP-, Citrate-), and successfully fermented most sugars except inositol and adonitol.

PCR was carried out for molecular detection of *E. coli*, targeting virulence gene *tsh* specific for APEC. Out of 24 sample, 17 (73.91%) were found to be positive and produced an amplicon of 153bp which was detected in ethidium bromide stained agarose gel. It showed Durg district had higher number of APEC positive in field isolates by PCR as compare to other districts. Nucleotide sequencing of DRG1 isolate were performed in the present study. It showed 98.23% homology with other sequences available in the NCBI GenBank. Phylogenetic analysis revealed that APEC/Durg/C.G. genomic sequence of DRG1 isolate of *E. coli* along with isolates of France and Norway might be sharing common ancestors.

Seventeen (70.83%) *E. coli* isolates produced dark coloured colonies with dry crystalline consistency on Congo red agar plate were considered positive biofilm formation.

Keywords: DRG1, *E. coli*, TSH, prevalence, pathology, PCR

Introduction

The Indian poultry sector is a rapidly growing industry that has transitioned into a highly organized sector since the 1960s. Currently, India ranks 3rd in egg production and 8th in poultry meat production globally. Poultry products are valued for their high protein, vitamin, and mineral content, as well as their affordability and religious acceptance. However, the intensive practices used to meet high demand have made poultry increasingly susceptible to infectious and metabolic diseases. Key management factors for health include a disease-free environment, proper sanitation, and adequate nutrition. Stressors like unhygienic conditions, overcrowding, and temperature extremes often lead to immunocompromise, allowing bacterial and viral infections to cause significant economic losses.

Avian Colibacillosis, caused by Avian pathogenic *Escherichia coli* (APEC), is a major global threat to the industry. Its most severe form, Colisepticaemia, is a systemic infection affecting multiple organs. Other manifestations include respiratory issues like air sacculitis, and reproductive infections such as salpingitis and egg peritonitis. These conditions result in high flock mortality and decreased egg production, leading to substantial financial impacts on both broiler and layer operations.

Avian Colibacillosis is caused by *Escherichia coli*, a Gram-negative bacterium and normal inhabitant of the poultry intestinal and respiratory tracts. Disease occurs when specific pathogenic strains, categorized as Avian Pathogenic *E. coli* (APEC), exploit host stressors such as immunosuppression or concurrent infections. APEC, a member of the extra-intestinal (ExPEC) group, causes significant morbidity, mortality, and economic losses, particularly in young chicks.

It often acts as an opportunistic pathogen triggered by environmental or viral factors. Transmission occurs horizontally through contaminated environment, feed, and water, or vertically via eggs. APEC also poses zoonotic risks and serves as a major reservoir for antimicrobial resistance.

Common lesions include colisepticaemia, omphalitis, coligranuloma, salpingitis, and egg peritonitis. Clinical signs vary from diarrhea to respiratory distress. Identification involves bacteriological and molecular techniques to detect virulence factors, such as Congo red binding and hemolytic activity. Standard isolation uses MacConkey and EMB agar, with suspected colonies confirmed by biochemical tests. Various virulence factors, including adhesins, hemolysins, and toxins, contribute to avian colibacillosis. Key genes include *tsh* for colonization, *pap* for adhesion, and *iss* for serum resistance. Globally, many *E. coli* serotypes are poultry pathogens; in India, O1, O2, and O78 are particularly significant. As Chhattisgarh lacks prevalence data and relies on traditional diagnostics, establishing a molecular diagnostic protocol is essential for disease control. Keeping the above facts into consideration, the present study was undertaken with the following objectives:

1. To study the prevalence of Colibacillosis in chickens of Durg, Rajnandgaon, Raipur and Surajpur districts of Chhattisgarh.
2. To study the pathomorphological alterations in various organs of affected chickens.
3. Molecular characterization of *E. coli* organism isolated from affected chickens.

Materials and Methods

The present study was undertaken for a period of 9 months from November 2021 to July 2022 in 20 poultry farms and the prevalence of avian Colibacillosis were recorded based on typical gross and microscopic lesions in chickens of Durg, Raipur, Rajnandgaon and Surajpur districts of Chhattisgarh.

$$\text{Prevalence (\%)} = \frac{\text{No. of birds showing gross lesions of Colibacillosis}}{\text{Total No. of birds examined}} \times 100$$

Pathological examination

Gross pathology

Whole carcasses collected from different poultry farms were routinely subjected to detailed and systemic necropsy examination, giving special attention to lesions in liver, heart, lungs, spleen, intestine, oviduct and yolk sac.

Histopathology

Tissue samples from various organs (liver, heart, lungs, spleen, intestine, and oviduct) of birds suspected of Colibacillosis were collected in 10% buffered formalin for histological examination. Specimens were sectioned to 0.5mm, washed overnight, and dehydrated through a graded acetone series. Following clearing with benzene and paraffin embedding, 3-5µm sections were cut, deparaffinized in xylene, and stained with Haematoxylin and Eosin (H&E) before mounting with DPX (Culling, 1963) [10]. Finally, the stained sections were examined under light microscopy.

Isolation and identification of *E. coli*

Isolation of bacteria: Swab samples from the internal organs of dead birds were inoculated in nutrient broth and

incubated at 37°C for 24 hours. The samples were then streaked onto MacConkey agar (MCA) and incubated for another 24 hours at 37°C. Pink colonies from MCA were subcultured on Eosin Methylene Blue (EMB) agar. Isolates displaying a metallic sheen on EMB were identified as *E. coli* and confirmed via biochemical characterization. Pure colonies were then subcultured on nutrient agar slants, incubated, and preserved at 4°C under sterile liquid paraffin for further study.

Molecular Characterization of *E. coli*

Samples for PCR

The isolates initially showing cultural features, morphological and biochemical characteristics pertaining to *E. coli* were subjected to PCR using avian pathogenic *E. coli* specific *tsh* gene primer (Subedi *et al.*, 2018) [39].

Extraction of DNA from *E. coli* isolates

Isolation of DNA for the PCR assay was prepared by heat lysis method proposed by (Choudhari *et al.*, 2020) [8] from *E. coli* isolates. The suspension of each isolates was made in 100 µl of milli-Q water by picking up a typical loopful colony of *E. coli* in a 200 µl PCR tube. Then vortexed and samples were heated at 95°C for 15 min. Then Centrifugation was done to remove cell debris at 10,000 rpm for 1 min and supernatant was used as a template DNA for PCR (Younis *et al.*, 2017 and Matin *et al.*, 2017) [45, 27].

Identification of APEC by PCR targeting *tsh* gene

APEC-specific primers targeting a 153 bp *E. coli* region were used (Ewers *et al.*, 2004) [17]. DNA was amplified via PCR following the protocol by Chaudhari *et al.* (2017) [7]. The 10 µl reaction mixture included 2 µl DNA, 0.4 µl of each primer, 5 µl TaqMixture, and 2.2 µl water. Thermal cycling involved initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 7 min. Products were stored at 4°C.

Table 1: Details of primer used in PCR to detect *tsh* gene of APEC

Primer	Sequence (5'-3')	Size
Forward	5'-TCTCAATGCGTCGTAACAGC-3'	153 bp
Reverse	5'-CCTTCAGATGAACGTCAGCA-3'	

Table 2: Composition of PCR reaction mixture

S. No.	Component	Volume
1.	2X PCR TaqMixture	5 µl
2.	Forward Primer (10 pmol)	0.4 µl
3.	Reverse Primer (10 pmol)	0.4 µl
4.	DNA template	2 µl
5.	Nuclease free water /PCR-grade water	2.2 µl
	Total volume	10 µl

Table 3: Thermal cycling conditions for detection of virulence gene *tsh* of APEC

S. No.	Steps	Temperature and time	Cycle
1.	Initial denaturation	94°C for 5 min.	35
2.	Denaturation	94°C for 30 sec.	
3.	Annealing	58°C for 30 sec.	
4.	Extension	72°C for 30 sec.	
5.	Final extension	72°C for 7 min.	
6.	Hold	4°C for 10 min.	

Agarose gel electrophoresis

Following PCR, amplified products were visualized via 1.5% agarose gel electrophoresis. Briefly, a gel casting tray was prepared with a comb and leveled. Agarose (0.75g) was dissolved in 50ml 1X TAE buffer, melted, and cooled before adding 5µl Ethidium bromide. The solution was poured into the tray and solidified for 30 minutes. After removing the comb, the gel was placed in an electrophoresis chamber filled with 1X TAE buffer, ensuring wells faced the cathode. PCR products (8µl) mixed with loading dye (2µl) and a 100bp DNA ladder were loaded into the wells. Electrophoresis was performed at 75V for approximately 45 minutes. Finally, the gel was visualized using a Gel Doc System.

Nucleotide Sequencing

Representative positive PCR products of the *tsh* gene were sequenced by Barcode Biosciences (Bengaluru) to generate consensus sequences. The resulting data, received as electropherograms and text files, were analyzed using NCBI BLAST to confirm specificity. Sequences were then compared with existing *E. coli* nucleotide data in GenBank (Dozois *et al.*, 2000) [14].

Phylogenetic analysis

The partial *tsh* gene sequences of APEC were compared with GenBank sequences. Nucleotide identities were aligned using MEGA 11 *Clustal W* and the Neighbour-joining method. Based on maximum identity scores, sequences were aligned to generate distances and construct a phylogenetic tree.

Results and Discussion

Prevalence of Avian Colibacillosis

Commercial poultry farms in Durg, Raipur, Rajnandgaon, and Surajpur districts of Chhattisgarh maintain large populations of broilers, cockerels, and layers. A study on Colibacillosis prevalence was conducted from November 2021 to July 2022. Post-mortem examinations were performed on 250 dead birds collected from 20 farms exhibiting clinical signs such as anorexia, respiratory distress, and diarrhea.

Out of the 20 farms surveyed, 12 were suspected of Colibacillosis based on gross lesions. Systematic post-mortem analysis revealed that 47 of the 250 birds (18.8%) showed lesions typical of the disease. The highest prevalence was recorded in Durg (25%), followed by Raipur (20.37%), Rajnandgaon (14.5%), and Surajpur (12%), as detailed in Table 4 and Graph 1.

Table 4: Prevalence (%) of avian Colibacillosis in different districts of Chhattisgarh

S. No	Districts	Poultry farms	Types of birds	Total no. of dead birds necropsied	Different pathological conditions caused by <i>E. coli</i>			Total no. of birds showing gross lesions of Colibacillosis	Prevalence (%)
					Colisepticaemia	Egg Peritonitis	Omphalitis		
1	Durg	Durg-1	Broiler	30	4	0	7	11	36.66%
		Durg-2	Broiler	15	1	0	2	3	20%
		Durg-3	Broiler	10	0	0	1	1	10%
		Durg-4	Layer	18	3	2	0	5	27.77%
		Durg-5	Layer	7	0	1	0	1	14.28%
		Durg-6	Broiler	4	0	0	0	0	0
		Sub Total (a)		84	8	3	10	21	25%
2	Rajnandgaon	Rjngn-1	Broiler	15	4	0	0	4	26.66%
		Rjngn-2	Broiler	12	3	0	0	3	25%
		Rjngn-3	Layer	9	0	0	0	0	0
		Rjngn-4	Broiler	15	2	0	0	2	13.33%
		Rjngn-5	Broiler	11	0	0	0	0	0
		Sub Total (b)		62	9	0	0	9	14.51%
		3	Raipur	Ripr-1	Broiler	15	0	0	1
Ripr-2	Broiler			18	5	0	2	7	38.88%
Ripr-3	Layer			10	2	1	0	3	30%
Ripr-4	Broiler			5	0	0	0	0	0
Ripr-5	Broiler			6	0	0	0	0	0
Sub Total(c)				54	7	1	3	11	20.37%
4	Surajpur			Srjpr-1	Broiler	13	0	0	0
		Srjpr-2	Broiler	17	4	0	2	6	35.29%
		Srjpr-3	Layer	9	0	0	0	0	0
		Srjpr-4	Broiler	12	0	0	0	0	0
		Sub Total (d)		50	4	0	2	6	12%
Total		(a+b+c+d)		250	28	4	15	47	18.8%

Rjngn - Rajnandgaon, Ripr - Raipur, Srjpr - Surajpur

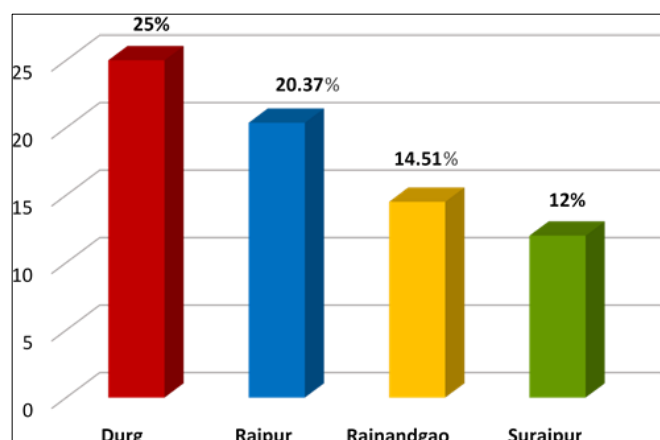
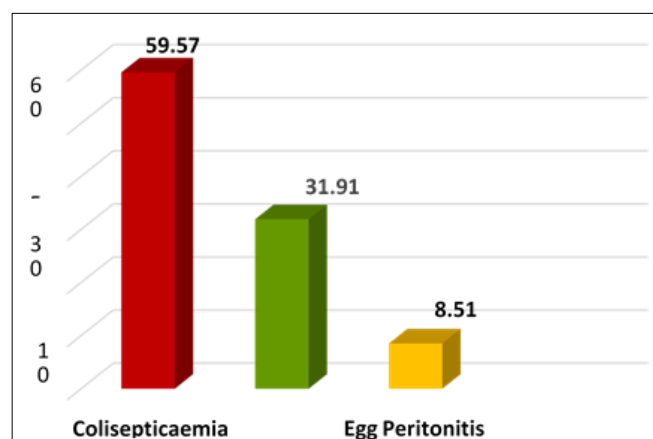
Note: Name of the farms is not being disclosed because of the fact that farm owners were reluctant to disclose the status of Colibacillosis in their farms.

Out of 47 *E. coli* infected birds, 28 (59.57%) birds showing suggestive lesions of Colisepticaemia, 15 (31.91%) showing

lesions of Omphalitis and 4 (8.511%) birds showing lesion of Egg Peritonitis (Table 5 and Graph 2).

Table 5: Prevalence of different disease conditions of *E. coli* infection in chickens

S. No	Disease conditions caused by <i>E. coli</i>	No. of birds showing gross lesions	Percentage (%)
1.	Colisepticaemia	28	59.57%
2.	Omphalitis	15	31.91%
3.	Egg Peritonitis	4	8.51%
Total no. of <i>E. coli</i> infected chickens		47	

**Graph 1:** Prevalence of avian Colibacillosis in different districts of Chhattisgarh**Graph 2:** Prevalence of disease conditions caused by *E. coli* infection

In this study, the overall prevalence of avian Colibacillosis was 18.8%, consistent with findings of 17.16% to 27.3% reported by Tawab *et al.* (2015) [41], Amin *et al.* (2017), and Boro *et al.* (2018) [6]. Conversely, several studies reported significantly higher rates: Khaton *et al.* (2008) [25] found (83–87%, while others recorded prevalence between (52.26% and 75.2% (Ahmed *et al.*, 2009; Dey *et al.*, 2013; Olarinmoye *et al.*, 2013; Yousef *et al.*, 2015) [3, 4, 13, 29, 46]. Jamoh *et al.* (2018) [23] reported up to 87.5% in broilers and 76.04% in layers. In India, Dadheech *et al.* (2016) [11] and Panchal *et al.* (2020) [31] also noted high prevalence, with the latter attributing a 71.38% rate to poor management. In contrast, Giasuddin *et al.* (2002) [22], Islam *et al.* (2004) [22], Omer *et al.* (2010) [30], and Surjagade *et al.* (2020) [40] reported much lower rates (3.65% to 6.56%), suggesting that superior hygienic practices reduce infection levels.

Pathological examination

Gross pathology

In this study, 250 post-mortem examinations were

performed, identifying 47 cases with lesions suggestive of Colibacillosis. Necropsies revealed *E. coli* infections such as Colisepticaemia, Egg Peritonitis, and Omphalitis. Gross lesions were primarily observed in the liver, heart, spleen, lungs, intestine, oviduct, and yolk sac, varying by disease condition.

Colisepticaemia

In the present study Colisepticaemia was observed in 59.57% of Colibacillosis affected chickens. Generally young broiler and layer chickens showed the characteristic lesions of Colisepticaemia. Liver, heart, lungs, spleen and intestine were carefully examined in Colisepticaemia affected birds.

a) Liver

The most consistent lesions in Colibacillosis-affected birds were observed in the liver. These included fibrinous perihepatitis characterized by white to yellowish exudate (Fig.3 and Fig.4), necrotic foci, and ascites (Fig.5). Mild cases showed hepatomegaly, congestion, and hemorrhages. These findings align with previous reports of Colibacillosis in poultry (Renu *et al.*, 2012; Tonu *et al.*, 2011; Kumar *et al.*, 2013; Shah *et al.*, 2019; Ahmad *et al.*, 2022) [33, 42, 26, 35, 2] and pigeons (Dutta *et al.*, 2013) [15]. Generalized visceral congestion likely resulted from bacterial endotoxins and vascular damage (Srinivasan *et al.*, 2014) [38].

b) Heart

Most chickens with Colisepticaemia exhibited typical fibrinous pericarditis. The pericardium was thickened, covered in thick fibrinous exudate, and adhered to the chest cavity. These findings align with observations by Khaton *et al.* (2008) [25], Tonu *et al.* (2011) [42], Shah *et al.* (2019) [35], and Surjagade *et al.* (2020) [40]. Similar lesions were reported by Nakamura *et al.* (1985) [28] in natural Colibacillosis outbreaks, while Ahmad *et al.* (2022) [2] also noted adhesive fibrinous perihepatitis and pericarditis in affected broilers.

Lungs, spleen and Intenstine: Affected birds showed splenic enlargement, oedema, congestion, and multifocal necrotic foci (Fig.7), with whitish fibrinous exudates in severe cases (Fig.8). These observations align with previous reports (Nakamura *et al.*, 1985; Srinivasan *et al.*, (2014) [28, 38]. Lungs exhibited congestion, haemorrhages, oedema, and focal fibrin deposits (Fig.9), consistent with findings by Gangane *et al.* (2006), Khaton *et al.* (2008) [25], and Kumar *et al.* (2013) [26]. Most birds displayed intestinal congestion, haemorrhages, and enteritis with thick yellowish exudate (Fig.10 and Fig.11). Severe cases presented fibrinous exudates on the gastrointestinal serosa and mesentery. These intestinal lesions correlate with studies by Khaton *et al.* (2008) [25], Tonu *et al.* (2011) [42], Srinivasan *et al.* (2013) [38], and Surjagade *et al.* (2020) [40], who noted similar mucinous exudate and congestion.



Fig 3: Showing fibrinous perihepatitis and pericarditis in broiler chicken affected with Colisepticaemia



Fig 4: Depicting fibrinous layer covering the surface of liver and heart of Colisepticaemia affected broiler chicken



Fig 5: Exhibiting congestion and yellowish fibrinous exudate on liver and heart along with ascites in chicken affected with colisepticaemia



Fig 6: Showing thick fibrinous covering over the pericardium in broiler chicken affected with colisepticaemia



Fig 7: Representing white necrotic foci (arrow), enlargement and congestion of spleen in Colisepticaemia affected broiler chicken



Fig 8: Showing fibrinous covering adhered over the surface of spleen in severe case of Colisepticaemia in chicken



Fig 9: Showing congestion, haemorrhages, oedema and focal fibrin deposits over the surface of lungs



Fig 10: Intestine showing enteritis along with excessive thick yellowish exudate in the lumen of Colisepticaemia affected chicken



Fig 11: Showing haemorrhages and yellowish exudate in the mucosal surface of intestine

Microscopic lesions

Tissue samples from birds showing gross lesions suggestive of *E. coli* infection were collected for histopathological examination. The tissue of liver, heart, lung and spleen were collected, processed and examined under microscope and finding were recorded.

Colisepticaemia

Histopathological analysis of the liver revealed thickened capsules with fibrin deposits and heterophil infiltration, characteristic of fibrinous perihepatitis (Fig. 12). Some instances also exhibited moderate fatty changes and Kupffer cell hyperplasia (Fig. 13). These findings align with

observations by Jamoh *et al.* (2018) [23] and Surjagade *et al.* (2020) [40].

Fibrinous perihepatitis may result from bacterial endotoxins and vascular injuries, which increase vascular permeability and allow fibrinogen leakage into tissues (Truscott *et al.*, 1974; Vegad, 2007) [43, 44]. Additionally, hepatic sinusoid dilatation, hepatocyte degeneration, Kupffer cell hyperplasia, congestion, and hemorrhages have been reported (Hooda *et al.*, 2011) [21].

In the heart, microscopic changes included pericardial thickening with fibrinous exudate, cellular infiltration, and necrosis (Fig. 14, 15). These findings align with the observations of Shrinivasan *et al.* (2014) [38] and Singh *et al.* (2018) [23, 36]. The spleen showed congestion, haemorrhages, lymphoid depletion, focal necrosis, and reticulo-endothelial cell proliferation (Fig. 16). These findings align with observations by Kumar *et al.* (2013) [26] and Singh *et al.* (2018) [23, 36], who also noted lymphocyte depletion in lymphoid organs during *E. coli* infection.

Lungs showed congestion, haemorrhages, and exudation in parabronchi, with dilated septae and perivascular exudate (Fig. 17). These lesions align with the findings of Abalaka *et al.* (2017) [1] and Shah *et al.* (2019) [35]. The intestine exhibited enteritis with mucosal epithelial desquamation and infiltration of heterophils and lymphocytes (Fig. 18). Degenerative changes, cryptal gland necrosis, and severe mononuclear cell infiltration were also observed (Fig. 19). Additionally, necrotic villi were heavily infiltrated with

inflammatory cells. These findings align with reports by Jan *et al.* (2018) [24] and Singh *et al.* (2018) [23, 36].

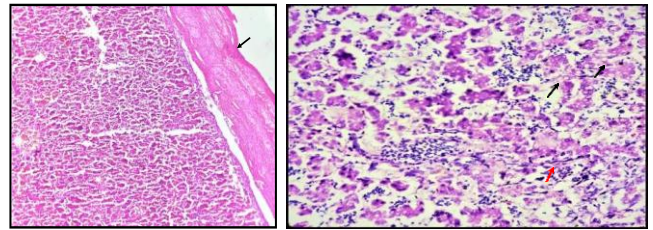


Fig 12: Fibrinous perihepatitis showing presence of large amount of fibrin deposits (arrow) along with infiltration of numerous heterophils in liver (H&E X100); Fig. 13. Section of liver depicting moderate fatty change (black arrows) along with Kupffer cell hyperplasia (red arrow) (H&E X400)

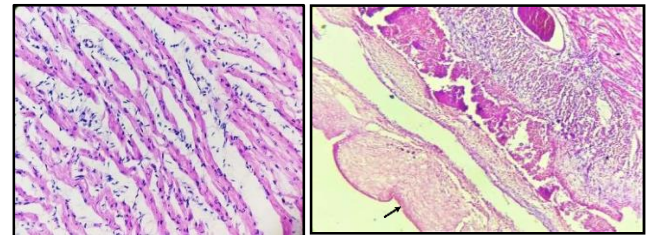


Fig 14: Section of heart representing fibrinous pericarditis (arrow) with infiltration of mononuclear cells (H&E X100); Fig. 15 Exhibiting severe degeneration of myocardial fibers and infiltration of mononuclear cells in heart (H&E X1000)

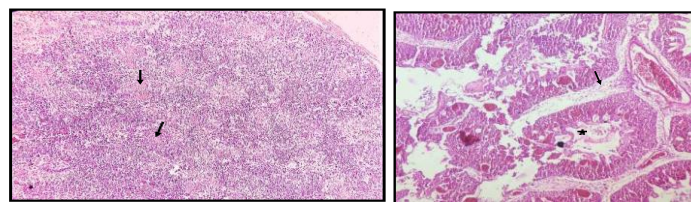


Fig 16: Section of spleen showing degeneration and necrosis (arrows) along with depletion of lymphoid follicles (H&E X 100); Fig. 17 Section of lung showing severe congestion, presence of exudate in the lumen of parabronchi (star) and dilatation of inter parabronchial septae (black arrow) (H&E X100).

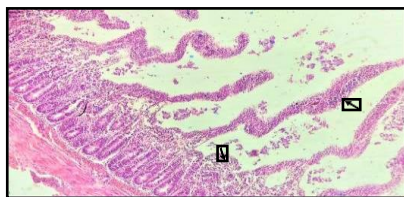


Fig 18: Section of intestine depicting desquamation, necrosis and infiltration of mononuclear cells (arrows) (H&E X100)

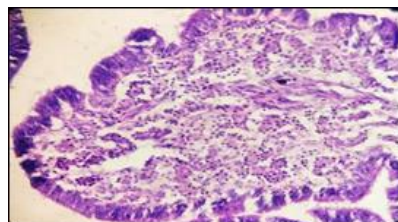


Fig 19: Section of oviduct showing degeneration and necrosis of epithelium of mucosal folds and severe infiltration of inflammatory cells (H&E X1000)

Isolation and Identification of *E. coli*

Isolation of *E. coli*

In this study, swab samples were collected from various organs of birds with lesions suggestive of Colibacillosis

across 12 broiler and layer farms. Based on cultural, morphological, and biochemical properties, 32 *E. coli* isolates (68.08%) were obtained from 47 cases, with 17 confirmed as APEC via PCR. The 68.08% isolation rate observed is comparable to the results reported by Ramasamy *et al.* (2008) and Sarkar *et al.* (2013).

Molecular detection of *E. coli* isolates

PCR of APEC targeting *tsh* gene

DNA from 24 EMB-positive *E. coli* isolates was screened for the APEC virulence gene *tsh* via PCR using specific primers (Table 1). Results showed that 17 isolates (73.91%) were positive, producing a 153bp amplicon (Fig. 20). This indicates a high prevalence of APEC in the Durg district, consistent with findings by Delicato *et al.* (2002) [12], Ewers *et al.* (2004) [17], and Chaudhari *et al.* (2017) [7]. Similar high detection rates were reported by Jan *et al.* (2018) [24], who found 80% positivity for *tsh*. Located on the ColV plasmid, *tsh* facilitates early-stage infection and visceral lesion development in chickens (Dozois *et al.*, 2000) [14]. Previous studies also link *tsh* presence to high-virulence APEC strains.

Nucleotide Sequencing

Nucleotide sequencing of positive PCR product of 1 isolate

(DRG1 isolated from Durg) was outsourced to Barcode Bioscience Pvt. Ltd, Bengaluru for *tsh* genomic sequence of APEC. Nucleotide sequencing of the DRG1 isolate revealed 153 bp sequence (Fig. 21). Electropherogram of *tsh* gene was examined (Fig. 22). BLAST (Basic Local Alignment

Search Tool) analysis showed that nucleotide sequence of PCR product of *tsh* gene of DRG1 isolate from Colibacillosis positive samples had 98.3% identity with other nucleotide sequences available in GenBank (<https://blast.ncbi.nlm.nih.gov>) (Table 6).

Table 6: Comparison of nucleotide sequences of *tsh* gene of DRG1 isolate of APEC with other similar sequences available in the GenBank

S. No	Strains of APEC	Accession Number	Origin	Identity (%)
1.	<i>Escherichia coli</i> strain BEN5202 genome assembly, plasmid: paAPEC5202	LR740759.1	France	98.23%
2.	<i>Escherichia coli</i> genome assembly FHI63, scaffold-26_contig-17.0_1_86934	LM996442.1	Norway	98.23%
3.	<i>Escherichia coli</i> strain ExPEC XM plasmid unnamed	CP025329.1	China 1	98.23%
4.	<i>Escherichia coli</i> strain ETEC 1766a <i>tsh</i> -like ETEC autotransporter (tleA) gene, complete cds	KF494347.1	Chile	98.23%
5.	<i>Escherichia coli</i> APEC O1 plasmid pAPEC-O1-ColBM, complete sequence	DQ381420.1	USA 1	98.23%
6.	69157 <i>Escherichia coli</i> strain QT598 plasmid pEC598, complete sequence	KP119165.1	Canada	98.23%
7.	<i>Escherichia coli</i> strain 11.4-R4 plasmid pCERC13, complete sequence	MK430046.1	Australia	98.23%
8.	<i>Escherichia coli</i> APEC O2-211 plasmid pAPEC-O2-211A-ColV, complete sequence	CP030791.1	Georgia	98.23%
9.	<i>Escherichia coli</i> chi7122 plasmid pAPEC-1, complete sequence	CP000836.1	USA 2	98.23%
10.	<i>Escherichia coli</i> strain H8Ecoli plasmid p3-H8	CP029214.1	China 2	98.23%
11.	<i>Escherichia coli</i> strain CFT073 chromosome	CP028309.1	Pakistan	98.23%
12.	<i>Escherichia coli</i> strain Ecol_244 plasmid pEC244_2, complete sequence	CP019018.1	United Kingdom	98.23%
13.	<i>Escherichia coli</i> strain MEI008 plasmid pMEI008_1, complete sequence	CP074681.1	Switzerland	98.23%
14.	<i>Escherichia coli</i> strain KMi025 plasmid pKMi025_1, complete sequence	CP091654.1	Japan	98.23%

Phylogenetic Analysis of *tsh* gene of APEC

The phylogenetic tree for the APEC/Durg/C.G. DRG1 isolate was constructed using MEGA 11 software. A total of 14 *tsh* gene sequences, including the DRG1 isolate and 13 accessions retrieved from GenBank (LR740759.1, LM996442.1, CP025329.1, KF494347.1, DQ381420.1, KP119165.1, MK430046.1, CP030791.1, CP000836.1,

CP029214.1, CP028309.1, CP019018.1, CP074681.1, and CP091654.1), were analyzed using Meg-align and Editseq algorithms. The analysis revealed two distinct clades (Fig. 23), showing that the local isolate is most similar to isolates from France and Norway. These results suggest that the DRG1 isolate shares a common ancestor with these European strains.

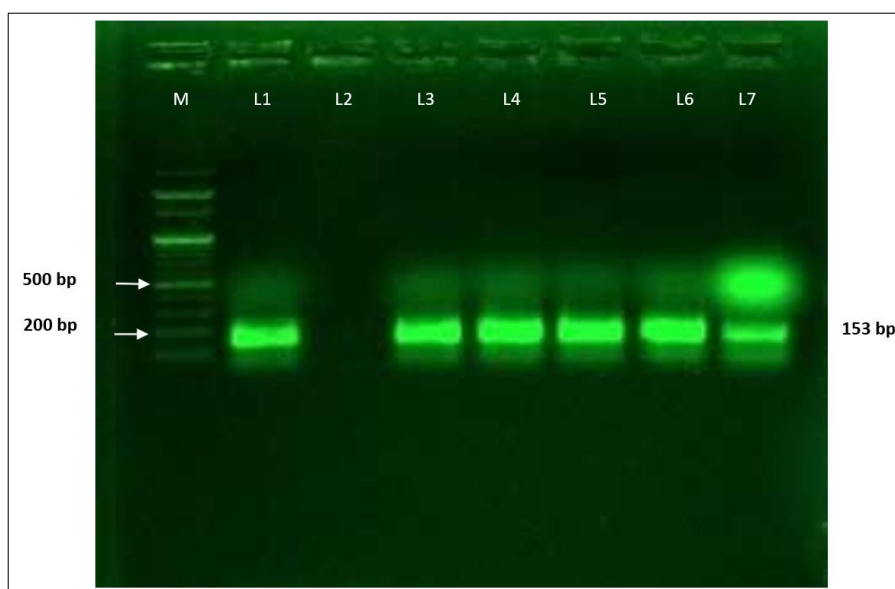


Fig 20: Detection of APEC using *tsh* gene specific PCR producing an amplicon of 153 bp Marker M: 100 bp DNA Ladder Lane L1: Positive control; Lane L2: Negative control; Lane L3,4,5,6,7: Positive samples of APEC.

```

5'CGGCGTTGTTTCCGGTAAAACCTTCAGTGGA
AGGACTGGAGTCTGACAGCCCGTGCCGGCCTGC
ATTATGAGTTCGATCTGACGGACAGTGCTGACG
GTTTCATCTGAAGGACTGTTGGCGGGCATCTGT
GAGTTTATTCTTTCTACCGTTGTGCTGACGTTCA
TCTGAAGGTTAATTAT3'

```

Fig 21: Partial nucleotide sequence of TSH gene of DGR1 isolate

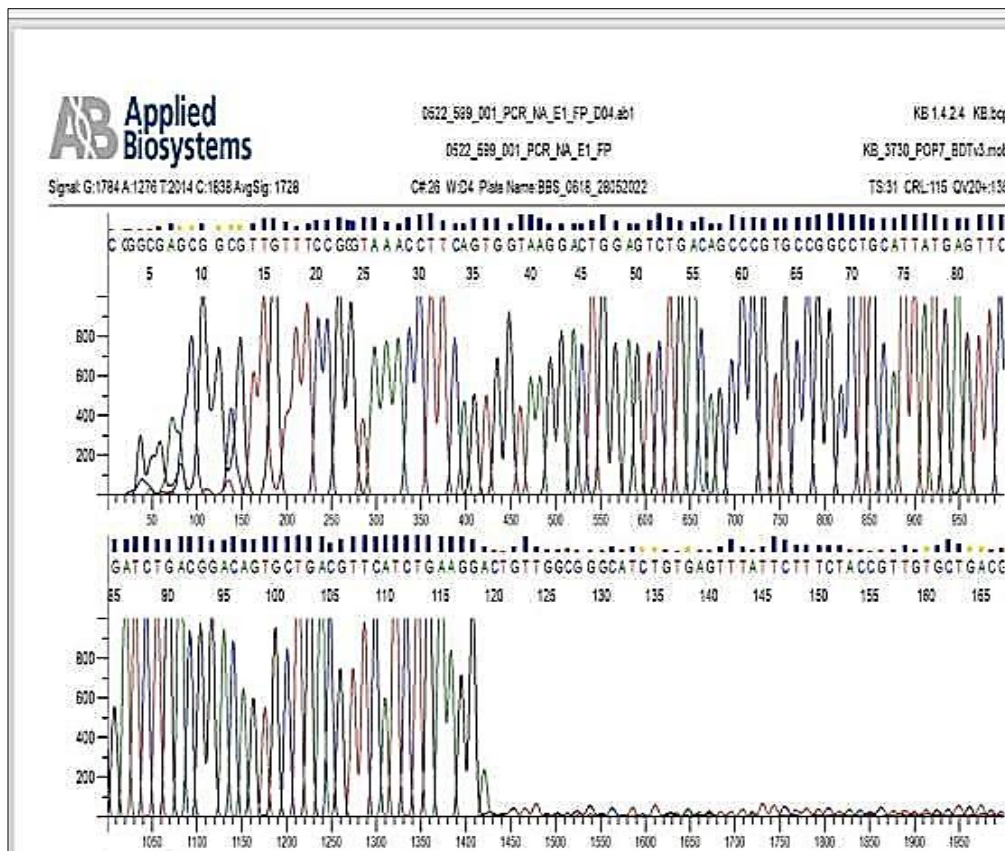


Fig 22: Electropherogram of tsh gene of APEC

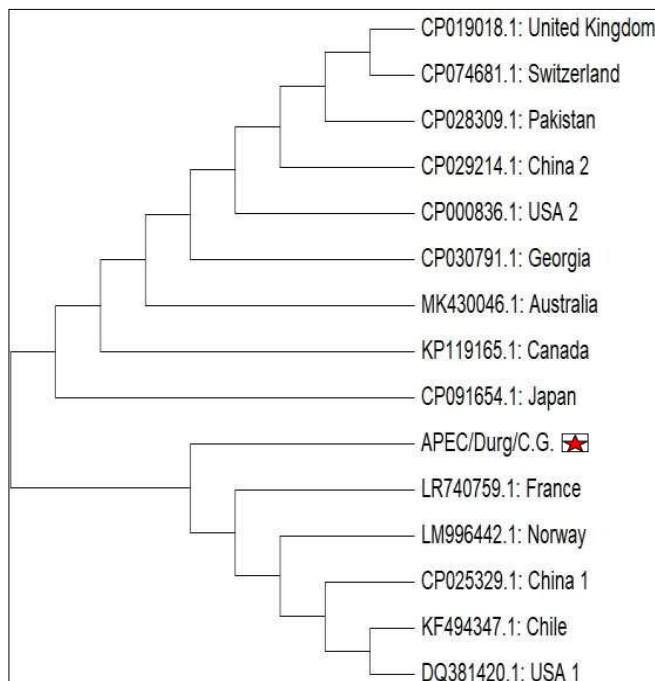


Fig 23: Phylogenetic tree showing relationship among APEC/Durg/C.G. along with other sequences retrieved from NCBI GenBank.

Conclusions

Over all prevalence of Colibacillosis in poultry farms was reported as 18.8% in Chhattisgarh. Highest prevalence was observed as 25% in Durg district followed by 20.37% in Raipur, 14.5% in Rajnandgaon and 12% in Surajpur districts of Chhattisgarh. Gross lesions in Colibacillosis affected chickens comprised of fibrinous perihepatitis, fibrinous

pericarditis, retained unabsorbed yolk in chicks, rupture of ovarian follicles and watery to yellowish yolk materials in peritoneal cavity in layer birds. Microscopic examination revealed fibrinous perihepatitis and pericarditis along with infiltration of inflammatory cells, degenerative, necrotic changes in liver, heart and spleen along with lymphocytic depletion in spleen. Partial genome sequence of APEC showed 98.23% similarities with isolates of France and Norway which showed that they had a common ancestor.

References

1. Abalaka SE, Sani NA, Idoko IS, Tenuche OZ, Oyelowo FO, Ejeh SA, Enem SI. Pathological changes associated with an outbreak of colibacillosis in a commercial broiler flock. J Vet Sci. 2017;15(3):95-102.
2. Ahmad T, Fiaz M, Sharif A, Nadeem M, Rehman SU, Rizwan M, Umer M. *In vitro* and *in vivo* evaluation of antimicrobials in *Escherichia coli* infection in broilers and evaluation of ciprofloxacin in induced colibacillosis. Pure Appl Biol. 2022;11(3):744-754.
3. Ahmed MD, Hashmi RA, Anjum AA, Hanif A, Ratyal RH. Drinking water quality by the use of congo red medium to differentiate between pathogenic and non-pathogenic *E. coli* at poultry farms. J Ani Plant Sci. 2009;19(2):108-110.
4. Ahmed MS, Sarker A, Rahman MM. Prevalence of infectious diseases of broiler chickens in Gazipur district. Bangladesh J Vet Med. 2009;7(2):326-331.
5. Amin U, Kamil SA, Shah SA, Dar TA, Mir MS, Ali R, Wani BM. Serotyping and prevalence of avian pathogenic *Escherichia coli* infection in broilers in Kashmir. Pharma Innovation Int J. 2017;6(10):336-338.
6. Boro SK, Pathak DC, Saikia GK, Buragohain M.

- Prevalence of colibacillosis in birds in and around Guwahati city (Assam). *J Entomo Zoo Stud*. 2018;6(1):1000-1003.
7. Chaudhari SV, Joshi BP, Desai DN, Bhandari BB, Choudhary KR, Madhwal A. Isolation and characterisation of *E. coli* infection from the bronchial plug of broiler birds associated with respiratory diseases. *Adv Ani Vet Sci*. 2017;5(8):334-341.
 8. Choudhari P, Ghodasara DJ, Kabariya DV, Bhandari BB, Momin SG. Isolation, antibiogram and molecular characterization of *E. coli* in broiler flocks. *Indian J Vet Sci Biotech*. 2020;16(4):35-39.
 9. Choudhary KK, Ghodasara DJ, Bhandari BB, Chaudhari SV, Madhwal A, Jani P. Etiopathological study on low pathogenic Avian Influenza complicated by *Escherichia coli* infection in layer birds. *International J Livestock Res*. 2020;10(11):44-52.
 10. Culling CFA. Handbook of histopathological and histochemical techniques: including museum techniques. 3rd ed. Butterworth-Heinemann; 1963. p. 405-557.
 11. Dadheech T, Vyas R, Rastogi V. Prevalence, bacteriology, pathogenesis and isolation of *E. coli* in sick layer chickens in Ajmer region of Rajasthan, India. *Int J Curr Microbio App Sci*. 2016;5(3):129-136.
 12. Delicato ER, Brito BG, Konopatzki AP, Gaziri LCJ, Vidotto M. Occurrence of the temperature-sensitive haemagglutinin among avian *Escherichia coli*. *Avian Dis*. 2002;46(3):713-716.
 13. Dey RK, Khatun MM, Islam MA, Hosain MS. Prevalence of multidrug resistant *Escherichia coli* in pigeon in Mymensingh, Bangladesh. *Microbes Health*. 2013;2(1):5-7.
 14. Dozois CM, Dho-Moulin M, Bree A, Fairbrother JM, Desautels C, Curtiss R. Relationship between the tsh autotransporter and pathogenicity of avian *Escherichia coli* and analysis of the genetic region. *American Society Microbio*. 2000;68(7):4145-4254.
 15. Dutta TK, Roychoudhury P, Bandyopadhyay SB, Wani SA, Hussain I. Detection and characterization of shiga toxin producing *Escherichia coli* (STEC) and enteropathogenic *Escherichia coli* (EPEC) in poultry birds with diarrhoea. *Indian J Med Res*. 2013;133(5):541-545.
 16. Government of India. Economic survey 2021-2022. New Delhi: Ministry of Finance; c2022.
 17. Ewers C, Janssen T, Kieslinga S, Philipp HC, Wielera LH. Molecular epidemiology of avian pathogenic *E. coli* (APEC) isolated from colisepticaemia in poultry. *Vet Microbio*. 2004;104:91-101.
 18. Freeman J, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. *J Clin Pathol*. 1989;42:872-874.
 19. Gangane GR, Kulkarni GB, Yeotikar PV. Studies on experimental colibacillosis in chicks. *Indian Vet J*. 2006;83:118-119.
 20. Giasuddin M, Sil BK, Alam J, Koike I, Islam MR, Rahman MM. Prevalence of poultry diseases in Bangladesh. *J Bio Sci*. 2002;2(4):212-213.
 21. Hooda A, Mishra SK, Nehra V, Lather D. Patho-anatomical studies on poultry with special reference to gastro-intestinal tract disorders. *Haryana Vet*. 2011;50:80-84.
 22. Islam MT, Islam MA, Samad MA, Kabir SM. Characterization and antibiogram of *Escherichia coli* associated with mortality in broiler and ducklings in Bangladesh. *Bangl J Vet Med*. 2004;2(1):9-14.
 23. Jamoh K, Rajkhowa TK, Singh YD, Ravindran R, Arya RS. Antimicrobial resistant *Escherichia coli* and associated colibacillosis in poultry population of Mizoram. *Indian J Vet Pathol*. 2018;42(3):185-190.
 24. Jan AW, Javed MT, Lone SQ, Rehman AU, Aslam MS, Javed A. Association of six selected pathogenicity genes of *E. coli* with gross and histopathological lesions in broiler chickens from field cases. *Pak J Agri Sci*. 2018;55(2):433-440.
 25. Khaton R, Haider MG, Paul PK, Das PM, Hossain MM. Colibacillosis in commercial chickens in Bangladesh. *Bangladesh Vet*. 2008;25(1):17-24.
 26. Kumar A, Bhalariao D, Gupta RP, Kumari M. Pathological studies on natural cases of avian colibacillosis in Haryana state. *Haryana Vet*. 2013;52:118-120.
 27. Matin MA, Islam MA, Khatun MM. Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. *Vet World*. 2017;10(1):29-33.
 28. Nakamura K, Yuasa N, Abe H, Narita M. Effect of infectious bursal disease virus on infections produced by *Escherichia coli* of high and low virulence in chickens. *Avian Pathol*. 1985;19(4):713-721.
 29. Olarinmoye AO, Oladele OO, Adediji AA, Ntiwunka UG, Tayo GO. Antibiograms of avian pathogenic *Escherichia coli* isolates from commercial layers with colibacillosis in Southwest Nigeria. *Malaysian J Microbio*. 2013;9(4):317-325.
 30. Omer MM, Abusalab SM, Gumaa MM, Mulla SA, Omer EA, Jeddah IE, AL-Hassan AM, Hussein MA, Ahmed AM. Outbreak of colibacillosis among broiler and layer flocks in intensive and semi intensive poultry farms in Kassala state, eastern Sudan. *Asian J Poultry Sci*. 2010;4(4):173-181.
 31. Panchal PP, Patel JM, Vihol PD, Raval JK, Patel DR, Danger N, Kalyani IH, Patel NM. Serotyping and prevalence of *Escherichia coli* infection in poultry of South Gujarat based on culture isolation and identification. *Int J Curr Microbio App Sci*. 2020;9(10):2512-2521.
 32. Ramasamy GKM, Dorairajan N, Balasubramaniam GA, Dinakaran AM, Saravanabava K. Pathogenic bacteria related to respiratory diseases in poultry with reference to *Ornithobacterium rhinotracheale* isolated in India. *Vet Arhiv*. 2008;78(2):131-140.
 33. Renu LMS, Pruthi AK, Mishra SK, Londhe MS, Deepika L, Anshu S. Etio-Pathological studies on poultry mortality with reference to *Escherichia coli* infections. *Indian J Poultry Sci*. 2012;47(2):222-226.
 34. Sarkar M, Roy JP, Batabyal K. Characterization and antibiogram of enteropathogenic *Escherichia coli* isolated from poultry. *Explor Ani Medi Res*. 2013;3(2):165-168.
 35. Shah SA, Mir MS, Wani BM, Kamil SA, Goswami P, Amin U, Shafi M, Rather MA, Beigh AB. Pathological studies on avian pathogenic *Escherichia coli* infection in broilers. *Pharma Innov*. 2019;8(7):68-73.
 36. Singh GK, Niyogi D, Tripathi KK, Joshi RK, Singh SV, Choudhary PK. Incidence of spontaneous *E. coli* infection in broiler chickens in Faizabad and Sultanpur districts of Uttar Pradesh. *Int J Curr Microbio App Sci*.

- 2018;7(5):5175-5181.
37. Srinivasan P, Balasubramaniam GA, Murthy TR, Balachandran P. Bacteriological and pathological studies of egg peritonitis in commercial layer chicken in Namakkal area. *Asian Pacific J Tropical Med*. 2013;3(12):988-994.
 38. Srinivasan P, Balasubramaniam GA, Murthy TR, Balachandran P. Pathomorphological studies of polyserositis in commercial caged layer chicken. *Asian Pacific J Tropic Medi*. 2014;7(1):313-320.
 39. Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S, Panthi P, Shrestha A, Chaudhary DK. Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* from broiler chickens in Chitwan, Nepal. *BMC Vet Res*. 2018;14(1):113.
 40. Surjagade S, Joshi BP, Mathakiya RA, Kapadiya B, Bansod AP, Jadhao AD, Prajapati R, Ghodasara DJ, Dave CJ, Jani P. Mortality pattern and pathological study of *E. coli* infection in broiler chickens. *J Entom Zoo Stud*. 2020;8(6):867-872.
 41. Tawab AA, Samir AEA, Ebtisam MM, Morsy DA. Prevalence of *E. coli* in broiler chickens in winter and summer seasons by application of PCR with its antibiogram pattern. *Benha Vet Med J*. 2015;29(2):119-128.
 42. Tonu NS, Sufian MA, Sarker S, Kamal MM, Rahman MH, Hossain MM. Pathological study on colibacillosis in chickens and detection of *Escherichia coli* by PCR. *Bangl J Vet Medi*. 2011;9(1):17-25.
 43. Truscott RB, Lopez AJ, Pettit JR. Studies of *Escherichia coli* infection in chickens. *Canadian J Comp Medi*. 1974;38:160-167.
 44. Vegad JL. A Textbook of Veterinary General Pathology. 2nd ed. Lucknow, UP: International Book Distributing Co.; 2007.
 45. Younis G, Awad A, Mohamed N. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic *E. coli* isolated from broiler chickens. *Vet World*. 2017;10(10):1167-1172.
 46. Yousef SA, Ammar AM, Ahmed DA. Serological and molecular typing of avian pathogenic *E. coli* originating from outbreaks of colibacillosis in chicken flocks. *International J Sci Res*. 2015;4(2):2319-7064.