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Association of Pathogenicity Genes (*cvaC*, *iss*, *iutA*, *Stx1A*, *Stx2A* and *Vat*) of *E. coli* with Gross and Histopathological Lesions of Colibacillosis in Broilers

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Abstract

Colibacillosis is an important disease of poultry caused by avian pathogenic *Escherichia coli* (APEC). The *E. coli* infection is associated with complications in chicken, which results in significant economic losses. For this purpose, study was conducted in the Faisalabad region of Pakistan. Study included the visits to the nearby poultry farms suspected of *E. coli* infections. The sampling was done from the farms and various diagnostic laboratories across Faisalabad region, including diagnostic laboratory University of Agriculture Faisalabad, Pakistan and district diagnostic laboratory. The main purpose of the study was to detect different pathogenicity genes (*iutA*, *iss*, *cvaC*, *stx1*, *stx2 and vat)* and associated lesions in broilers. Morbid organs including heart, liver, spleen, intestine, etc. of clinically positive birds were collected for histopathological studies and culture isolation. For histopathological study, tissues were preserved in 10% formalin. Culturing was done on MacConkey agar and the positive growth was confirmed on triple sugar iron agar. DNA was extracted from bacterial colonies. The gene was detected on different sample infected with *E. coli* through PCR. The results concluded that the prevalence of *iutA* genes was detected @ 96%, *iss* 88%, *cvaC* 64%, *stx1* and *stx2* @ 0%, while *vat* was detected 40% on different organs of broilers, respectively.

Introduction

In Pakistan, poultry meat has 28% share in total meat production. There is a wide range of infectious diseases which affect the productive performance of the birds. Among infectious diseases, bacterial diseases are more common in birds [1]. These infectious diseases cause economic losses in poultry industry in terms of high mortality, morbidity and decrease in production [2]. Colibacillosis is one of the important bacterial diseases which cause lesions in different systems of the body in chicken [3]. In poultry, *Escherichia coli* infection also causes cystic degeneration of ovarian follicles, cellulites, enterocolitis, acute septicemia, panopthalmitis, arthritis and osteomyelitis. The *Escherichia coli* infection in chicks can occur by a number of routes. Eggs contract *Escherichia coli* infection mainly from the contaminated fecal matter and it is considered as the main route of *Escherichia coli* infections in newly hatched chicks [4]. Diseases such as Salmonellosis, Aspergillosis and Colibacillosis tend to increase mortality from 0.3 to 3% in the first one or two weeks after hatching. However, all the strains of *Escherichia coli* are not pathogenic in nature. Some strains of the *Escherichia coli* are natural inhabitants of the gut flora in the poultry birds. Various serological and bacteriological tests have been used over the years to distinguish between pathological and non-pathological strains of *Escherichia coli.*

Several studies have been conducted in an attempt to understand the pathogenic mechanisms and virulence factors expressed by the pathogenic strains of *Escherichia coli* [5]. Genomic investigations for virulence factors of *E. coli* are more effective and considered more reliable in comparison with other tests used. Genomic methods have globally provided very interesting results [6]. Heat-stable (ST) and heat labile (LT), along with shiga toxins (*stx1A* and *stx2A*) and vacuolating auto-transporter toxin (*vat*) have been identified in avian pathogenic *E. coli* [7]. Identification of *Escherichia coli* strains as Avian Pathogenic *Escherichia coli* (APEC) isolates is pre-requisite if flock specific vaccines are to be produced. The genotyping methods are more preferred and are considered reliable than serotyping. Genotyping not only detects the virulence associated genes but also differentiate between pathogenic and non-pathogenic bacteria. By using PCR, it is possible to detect virulence associated (*cvaC, iss, iutA*) [8] and toxicity genes (*stx1A, stx2A, vat*) [9]. The virulent genes like *cvaC, iss* and *iutA*, were detected at the rate of 35.5, 38.5 and 63.0 percent, respectively from the cases of early chick mortality [8]. The toxic genes (*stx1A, stx2A, vat*) were reported at the rate of 0.0, 0.0 and 14.8 percent, respectively [9]. A considerable literature is available about the identification of the pathogenicity genes from *Escherichia coli* isolated from the field cases. However, no information is available from local isolates about pathogenicity genes in strains of *Escherichia coli* causing mortality in broiler, in Pakistan and their association with pathology. Therefore, a study was planned to detect the six virulence associated genes in APEC in local isolates, and to find out the association of these virulent genes with gross and histopathological lesions in broilers.

Materials and Methods

Sample Collection

The sampling was done from sick and freshly dead birds, (broiler) coming to the Diagnostic laboratory, Department of Pathology, Faculty of Veterinary Science, University of Agriculture Faisalabad, Central Poultry Diagnostic Laboratory Faisalabad and Central Veterinary Hospital Faisalabad. Information about each farm was recorded. The morbid organs including liver, spleen, heart, intestine, lung, etc. from dead birds were collected into two different containers. A portion of the organ was collected without formalin for culture isolation and the other was collected and fixed in formalin for histopathology.

Histopathology

Tissues including liver, spleen, heart, intestine, lung, etc., were processed for histopathological studies and slide preparation following the method as described by Bancroft and Gamble (2008) [10].

Culture test

Culture isolation was done on nutrient agar, MacConkey agar and TSI agar [11]. Colony characteristics were noted [12].

Molecular Detection

DNA was obtained from bacterial colonies obtained on culture media, using boiling method as described previously [13]. Six different pathogenicity genes of *E. coli* (as mentioned in Table 1) were targeted for amplification using forward and reverse primers. Amplified products were separated and visualized using ethidium bromide gel documentation system (Bio-Rad).

Table: 1 Primers sequence

Primer	Primer sequence (5' – 3')	Genes	Reference
cvaC f	CACACACAAACGGGAGCTGT	cvaC	Johnson *et al*., 2000
cvaC r	CTTCCCGCAGCATAGTTCCAT	cvaC	
Iss f	CAGCAACCCGAACCACTTGATG	Iss	Johnson *et al*., 2008
Iss r	AGCATTGCCAGAGCGGCAGAA	Iss	
iutA f	GGCTGGACATCATGGGAACTGG	iutA	Johnson *et al*., 2000
iutA r	CGTCGGGAACGGGTAGAATCG	iutA	
Vat f	TCCTGGGACATAATGGTCAG	Vat	Ewers *et al*., 2004
Vat r	GTGTCAGAACGGAATTGT	Vat	
Stx1Af	CAGTTAATGTGGTGGCGAAG	Stx1A	Jeong *et al*., 2012
Stx1A r	CTGCTAATAGTTCTGCGCATC	Stx1A	
Stx2A f	CTTCGGTATCCTATTCCCGG	Stx2A	Jeong *et al*., 2012
Stx2A r	GGATGCATCTCTGGTCATTG	Stx2A	

Results

General Observations

The history record of the samples revealed that 80% of the farms were open houses, while 20% were controlled houses. It also revealed that 36% of the flocks were treated and 64% were not treated by self-medication. The history record showed that 36% of the flocks had normal appetite, while 64% of the flocks were reported to have low appetite. The history record revealed that 56% of the flocks had *E. coli* problems in birds aged 10-25 days, with body weight between 500-1000 gm and 100% mortality on the farms.

Postmortem Findings:

The overall percentage of gross lesion seen in each organ is presented in **Table 2**. The gross lesions on liver, heart, lungs, spleen and intestine were classified as mild, moderate and severe. The results revealed that gross lesions in liver of perihepatitis were identified in 96% dead birds, out of which 20% showed mild, 20% moderate and 56% severe lesions. The pericarditis was also identified in 92% of dead birds, out of which 4% showed mild, 48% moderate and 40% severe. The lungs were found congested in 84% cases, out of which 16% shown mild, 12% moderate and 56% severe. The spleen appeared enlarged, congested and hemorrhages were also seen. The changes in spleen were seen in 84% cases, out of which 28% showed mild, 20% moderate and 36% severe changes. Other lesions recorded were pale kidneys, proventriculus showed dark color, air sacs were thickened and cloudy, congested muscle and congested and hemorrhagic cecal tonsils. The kidney lesions were seen in 24% cases, gizzard lesions in 32% cases and proventriculus lesions in 32% cases, air sacculitis in 88% cases and tracheal lesions in 72% cases. The lesions were seen in 16% cases of bursa Fabricious.

Table 2: Identification of gross and microscopic lesion in different organ

Organs	Gross lesions	Histopathological lesions
Liver	96%	96%
Lungs	84%	80%
Heart	92%	85%
Kidney	96%	72%
Spleen	84%	84%
Intestine	88%	88%

Histopathological Findings:

The overall percentage of histopathological lesions seen in each organ are presented in Table: 2. The histopathological results revealed that in 16% cases microscopic lesions in liver were mild, in 24% moderate and in 56% were severe. In lungs, in 36% cases lesions were mild, 28% were moderate and 16% were severe. The heart lesions showed that in 20 % cases were mild, 25% were moderate and 40% were severe. In kidneys, in 36% cases were mild, in 32% cases were moderate and in 4% cases were severe. In spleen, in 32% cases were mild, in 24% were moderate and in 28% cases were severe. In intestine, in 16% cases were mild, in 36% cases were moderate and in 36% cases were severe.

The histopathological changes in liver were inflammation in portal triad area, atrophied hepatic cords and dilated sinusoidal spaces. The changes in heart muscles were degeneration of muscle fibers, with loss of cross striations, condensation of some nuclei and mild inflammatory reaction. The changes in kidney were necrosis in renal tubular epithelial cells with condensed nucleus and more eosinophilic cytoplasm and in some cases vacuolar changes were present in the cytoplasm. The epithelial cells were also seen fallen in the lumen of tubules. In intestine, epithelium appeared sloughed off, villi appeared broken and mild to moderate inflammatory changes were evident in deeper layers of intestine, i. e., submucosa (**Figure 1**).

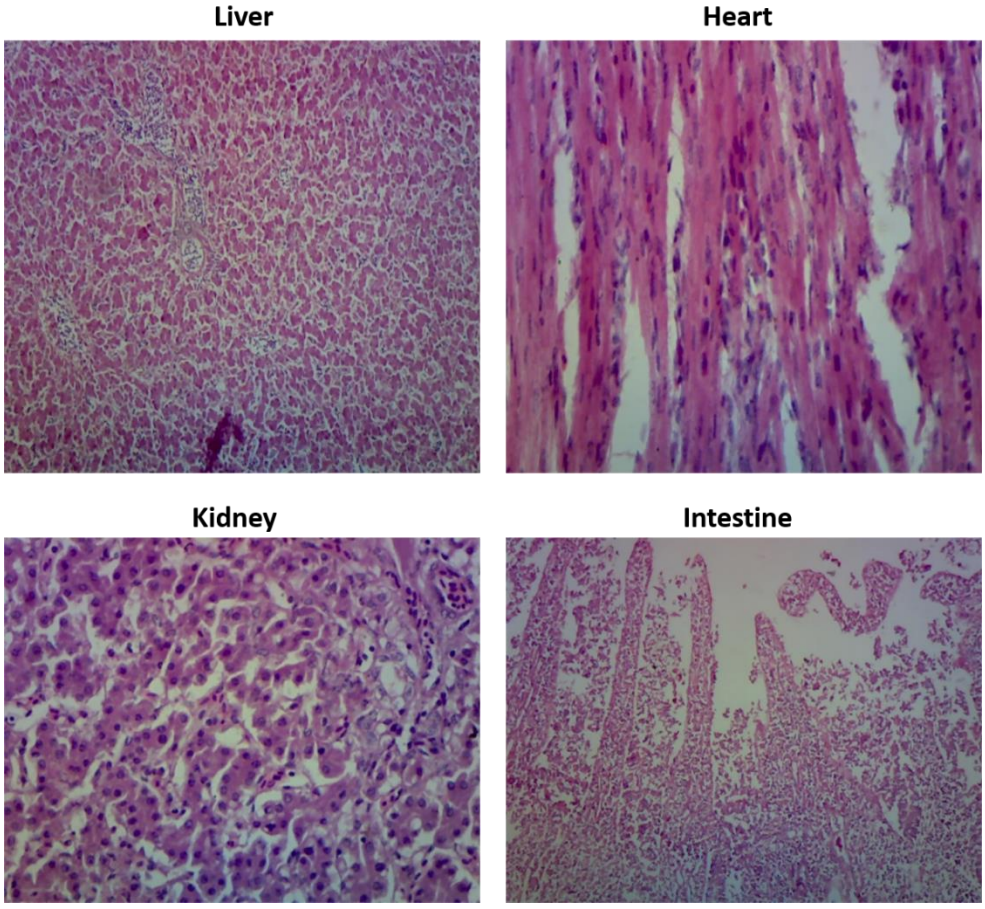
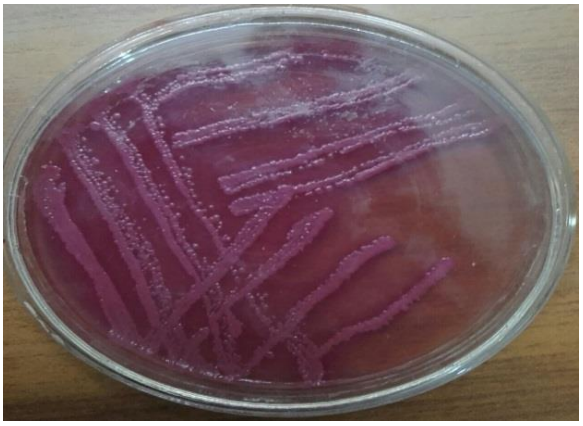


Figure 1: Pathological analysis of lesions in Liver, Heart, Kidney and Intestine

Isolation of pure bacteria and PCR

A total of 25 samples were cultured on MacConkey agar and all samples were cultured positive on MacConkey agar. There were pink colonies of *Escherichia. coli.* The positive colonies were further inoculated on TSI agar and the samples growth appeared on TSI was yellow colored colonies.



***E. coli* isolates showing rose pink colored colonies on MacConkey agar**

The results of PCR for the pathogenic genes of *Escherichia coli* is shown in **Table 3**. Results showed that genes *iutA, iss, cvaC and vat* were positive at the rate of 96%, 88%, 64%, and 40%, respectively. However, the *stx1* and *stx2* genes were not detected from the *E. coli* isolates.

Table 3: The results of PCR of pathogenicity genes are presented

Parameters	Frequency	Percentage (%)	C.I
iutA gene	24	96	81.81 to 99.80
Iss	22	88	70.72 to 96.86
cvaC	16	64	44.11 to 80.81
Vat	10	40	22.41 to 59.79
Stx1	0	0	0.00 to 11.29
Stx2	0	0	0.00 to 11.29

Association of pathogenicity genes cvaC, Iss, iutA, Vat, Stx1, Stx2 with Gross and Histopathological lesions

The relationship between *iutA, iss, cvaC, stx1, stx2 and vat* genes with microscopic lesions of liver, heart,

spleen and intestine infected with *E. coli* are presented in **Table 4**. Results showed that *cvaC* gene was present in 64%, *iss* gene was present in 88%, *iutA* gene was present in 96%, *vat* was present in 40%, *stx1* and *stx2* genes were present in 0% of microscopic lesions.

Table 4: Association of *cvaC, iss, iutA, vat, stx1 and stx2* genes with severity of microscopic lesions in liver, heart, spleen and intestine

	Severity of lesions				
	No lesions (%)	**Mild (%)**	**Moderate (%)**	**Severe (%)**	**Total (%)**
Liver					
cvaC					
Not detected	1	1	4	3	9(36)
Detected	0	3(12)	2(8)	11(44)	16 (64)
Iss					
Not detected	0	1	1	1	3(12)
Detected	1(4)	3(12)	5(20)	13(52)	22(88)
iutA					
Not detected	0	1	0	0	1(4)
Detected	1(4)	3(12)	6(24)	14(56)	24(96)
Vat					
Not detected	1	1	4	9	15(60)
Detected	0	3(12)	2(8)	5(20)	10(40)
Stx1					
Not detected	1	4	6	14	25(100)
Detected	0	0	0	0	0
Stx2					
Not detected	1	4	6	14	25(100)
Detected	0	0	0	0	0
Heart					
cvaC					
Not detected	2	0	5	2	9(36)
Detected	0	2 (8)	5(20)	9(36)	16(64)
Iss					
Not detected	1	0	2	0	3(12)
Detected	1(8)	2 (8)	8(32)	11(44)	22(88)
iutA					
Not detected	0	0	1	0	1(4)
Detected	2 (8)	2 (8)	9(36)	11(44)	24(96)
Vat					
Not detected	2	0	7	6	15(60)
Detected	0	2 (8)	3(12)	5(20)	10(40)
Stx1					
Not detected	2	2	10	11	25(100)
Detected	0	0	0	0	0
Stx2					
Not detected	2	2	10	11	25(100)
Detected	0	0	0	0	0
Spleen					
cvaC					
Not detected	2	4	2	1	9(36)
Detected	2(8)	4(16)	4(16)	6(24)	16(64)
Iss					
Not detected	2	0	0	1	3(12)
Detected	2(8)	8(32)	6(24)	6(24)	22(88)
iutA					
Not detected	1	0	0	0	1(4)
Detected	3(12)	8(32)	6(24)	7(28)	24(96)
Vat					
Not detected	3	4	4	4	15(60)
Detected	1(4)	4(16)	2(8)	3(12)	10(40)
Stx1					
Not detected	4	8	6	7	25(100)
Detected	0	0	0	0	0
Stx2					
Not detected	4	8	6	7	25(100)
Detected	0	0	0	0	0
Intestine					
cvaC					
Not detected	2	1	5	1	9(36)
Detected	1(4)	3(12)	4(16)	8(32)	16(64)
Iss					
Not detected	2	1	0	0	3(12)
Detected	1(4)	3(12)	9(36)	9(36)	22(88)
iutA					
Not detected	1	0	0	0	1(4)
Detected	2(8)	4 (16)	9(36)	9(36)	24(96)
Vat					
Not detected	2	1	6	6	15(60)
Detected	1(4)	3(12)	3(12)	3(12)	10(40)
Stx1					
Not detected	3	4	9	9	25(100)
Detected	0	0	0	0	0
Stx2					
Not detected	3	4	9	9	25(100)
Detected	0	0	0	0	0

Strains identified on the basis of presence or absence of six genes

The strains were identified on the basis of presence or absence of six genes are shown in **Table 5** and **Figure 2**. The strains *ECS1* was present on 10 farms, *ECS2* on 6 farms, *ECS3* on 6 farms and *ECS4* on 2 farms, respectively.

Table: 5 Strains identified on the basis of presence

Strain	Number of farms	Identified genes
ECS1	10	cvaC, iss, iutA,vat
ECS2	6	cvaC, Iss, iutA
ECS3	6	Iss, iutA
ECS4	2	iutA
ECS5	0	No gene

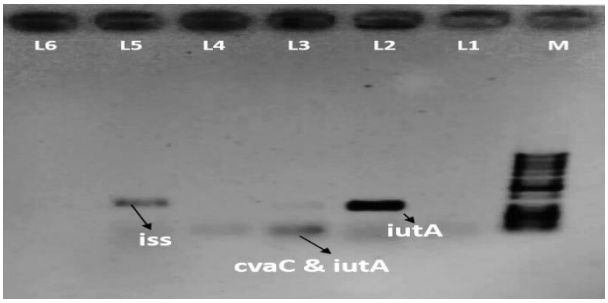


Figure 2: PCR identification of different *E. coli* strains on the basis of presence or absence of six genes

Discussion

Among the bacterial diseases, colibacillosis is one of the important infectious diseases which causes lesions in different systems of the body and affect the productive-performance of the poultry birds. Colibacillosis is caused by avian pathogenic *Escherichia coli* (APEC). They produce lesions in different parts of the body such as peritonitis, pericarditis, air sacculitis, perihepatitis, osteomyelitis, omphalitis, synovitis and also cause thickness of epicardium and pericardium due to accumulation of exudate which is fibrinous in nature [14]. The organisms of *E. coli* are divided into pathogenic and non-pathogenic, based on their ability to cause disease. The virulent genes *iutA, iss* and *cvaC* are associated with pathogenic *E. coli.* In the previous study, the MacConkey agar medium was used for the identification and isolation of bacteria because it is lactose fermenters growth of gram-negative bacteria [11].

We observed the pinkish brick colored colonies those appeared after 24-48 hrs. We investigated the association of selected six pathogenic and toxic genes (*iutA, iss, cvaC, stx1, stx2 and vat*) of *E. coli* with gross lesions and histopathological lesions in broilers. We identified these six genes through PCR. Our results revealed that the prevalence of toxic gene *iutA* was high as compared to other toxic genes (*iss* and *cvaC*). However, *Vat* gene was observed in 40% cases, while the gene of shiga toxins (*stx1A* and *stx2A*) were not identified in local isolates. In the previous study, it has been stated that the frequency of toxic gene *iutA* was more as compared to others seven toxic genes (Delicato *et al.,* 2003). In our study the results strongly agreed with the previous results because it was also isolated with more frequency (*iutA)* as compared to other genes [15].

In the previous study the *E. coli* produced microscopic lesions as observed in different organs. It was also observed that some organs showed mild, some showed moderate and some showed severe changes after *E. coli* infection [14, 16]. We also observed the liver infected with *E. coli* showed microscopic lesions. There were 16% mild, 24% moderate and 56% severe lesions. Spleen showed 32% mild, 24% moderate, 28% severe lesions and intestine showed 16% mild, 36% moderate and 36% severe histopathological lesions. Liver histopathology showed inflammation, atrophied hepatic cords, and dilated sinusoidal spaces. The heart section showed degenerative changes in the muscle along with loss of cross striations. The intestine section showed villi appeared broken, necrotic and inflammatory changes in the intestine with sloughing of epithelium. The present study results corresponded with the findings of others [14, 16].

In the previous study toxic genes were identified through PCR and their results were stated that the toxic gene *iutA* was identified in 71%, *iss* in 42% and *cvaC* in 35% cases from infected birds [17]. In the present study the three toxic genes were identified through PCR from different organs of the broilers infected with *E. coli*. We observed that the toxic gene of *iutA* gene was identified in 96% of cases of poultry birds which showed microscopic changes in the liver, while *iss* gene was identified in 88% cases and *cvaC* was identified in 64% cases from different organs (liver, heart, spleen, kidney and intestine) of broilers.

The toxigenic genes like *stx1, stx2* and *vat* can cause disease in poultry. *E. coli* (EHEC) is important to the production of Shiga toxin (Stx) which is absorbed into blood and damage the systemic vasculature resulting in edema disease and hemorrhagic colitis [18]. It was stated that the toxigenic genes were not identified from any organs of broilers in Korea [9]. In the present study we investigated the toxigenic genes from different infected organs of the poultry. The present results revealed that a total number of 5 strains of *E. coli* were identified on the basis of presence or absence of six genes at 25 farms. One strain ECS1 was present at 10 farms, in which *cvaC, iss, iutA* and *vat* were identified. ECS2 was present at 6 farms in which *cvaC, iss, iutA* genes were identified. ECS3 was present at 6 farms in which *iss* and *iutA* gene were identified. ECS4 was present at 2 farms in which *iutA* gene was identified. The strain ECS5 was present at one farm in which no gene was identified.

Conclusions

The APEC pathogenicity related four genes from a set of six could be identified in local *E. coli* isolates. There is significant relationship between four pathogenicity genes of *E. coli* with gross and histopathological lesions of liver, heart, spleen and intestine. By using PCR, it is possible to detect virulence associated (*cvaC, iss, iutA*) and toxicity genes (*stx1A, stx2A, vat*) (Jeong *et al.*, 2012). The virulent genes like *cvaC, iss* and *iutA*, were detected at the rate of 35.5, 38.5 and 63.0 percent, respectively from the cases of early chick mortality [19]. The toxic genes (*stx1A, stx2A, vat*) were reported at the rate of 0.0, 0.0 and 14.8 percent, respectively [9].

Conflict of Interest

All authors have disclosed no conflicts of interest.

References

[1] Ahmad, I., M.S. Anjum and M. Hanif. 2012. Prevalence of poultry diseases at high altitudes of District Poonch Azad Jammu and Kashmir. J. Pak. Sci. 64: 334-336.

[2] Alexander, D.J. 2000. The History of Avian Influenza in World Poultry. Elsevier. 13: 7-8.

[3] Rodriguez, S.C., W. Giddings, C. Doetkott, T. J. Johnson and L.K. Nolan. 2004. Characterizing the APEC pathotype. Vet. Res. 36: 241–256.

[4] Nolan, L.K., H.G. Barnes, J. Waillencourt, T. Abdul Aziz, C.M. longue, D.E. Swayne, L.R. Dougald, D.L. Knolam and V. Nair. 2013. Collibacillosis in Diseases of Poultry. Am. Assoc. Avian Path. pp: 751-805.

[5] JanBen, T.C., P. Schwarzi, M. Preikschati, H.C. Philipp and L.H. Wieler. 2001. Virulence associated genes in avian pathogenic Escherichia coli (APEC) isolated from internal organs of poultry having died from colibacillosis. J. Med. Microbiol. 291: 371-378.

[6] Dho, M.M., J.M. Fairbrother. 1999. Avian pathogenic Escherichia coli. Vet. Res. Biom. Cent. 30: 299-316.

[7] Parreira, V.R., and C.L. Gyles. 2003. A novel pathogenicity island integrated adjacent to the tRNA gene of avian pathogenic Escherichia coli encodes a vacuolating autotransporter toxin. Infect. Immun. 71: 5087-5096.

[8] Johnson, T.J., Y. Wannemuehler, C. Doetkott, S. J. Johnson, S.C. Rosenberger and L.K. Nolan. 2008. Identification of minimal predictors of avian pathogenic Escherichia coli virulence for use as a rapid diagnostic tool. J. Clin. Microbiol. 46: 3987-3996.

[9] Jeong, Y.W., T.E. Kim, J.H. Kim and H.J. Kwon. 2012. Pathotyping avian pathogenic Escherichia coli strains in Korea. J. Vet. Sci. 13: 145-152.

[10] Bancroft, J.D. and M. Gamble. 2008. Theory and Practise of Histopathological Techniques, 2nd ed. Edinburgh: Churchill Livingstone Publication. 43: 85-100.

[11] Tonu, N.S., M.A. Sufian, S. Sarker, M.M. Kamal, M.H Rahman and M.M. Hossain. 2011. Pathological study on colibacillosis in chickens and detection of Escherichia coli by PCR. J. Vet. Med. 9: 17 - 25.

[12] Carter, G.R. 1986. Essentials of Veterinary Bacteriology and Mycology, 3rd ed. pp: 312-330.

[13] Mazaheri, S., S.S. Ahrabi and M.M. Aslani. 2014. Shiga toxin-producing Escherichia coli isolated from lettuce samples in Tehran, Iran. J. Microbiol. 7: 12346.

[14] Kumar, D.A. Bhalerao, R.P. Gupta and M. Kumari. 2013. Pathological studies on natural cases of avian colibacillosis in haryana state. Haryana Vet. J. 52: 118-120.

[15] Delicato, E.R., B.D. Brito, L.J. Gaziri and M.C. Vidotto. 2003. Virulence-associated genes in Escherichia coli isolates from poultry. J. Vet. Microbiol. 94: 97–103.

[16] Ghosh, R.C., S.D. Hirpurkar and P.R. Suryawnshi. 2006. Concurrent colibacillosis and infectious bursal disease in broiler chicks. Indian. Vet. J. 83: 1019-1020.

[17] Mototaka, H., U. Masaru, A. Tetsuo, M. Kawanishi, T. Mai, S. Imamura, S. Hideto, A. Kojima and T. Asai. 2014. Phylogenetic grouping epidemiological typing analysis of virulence genes and antimicrobial susceptibility of Escherichia coli isolated from healthy broilers in Japan. Irish Vet. J. 67:14-23.

[18] Soon, G.K., S.E. Yeoun, C.E.J. Choi, K. Bokyung, S.H. Jong, K.J. Hyug. 2008. Epidemiological prevalence of Avian Pathogenic Escherichia coli Differentiated by Multiplex PCR from commercial chickens and Hatchary in Korea. J. Bacteriol. Virol. 38: 179-188.

[19] Johson, J.R., A.L. Stell, F. Scheutz, T.T. Bryan, T.A. Russo, U.B. Carlino, C. Fasching, J. Kavle, L.V. Dijk and W, Gaastra. 2000. Analysis of the F antigen-specific papA alleles of extraintestinal pathogenic Escherichia coli using a novel multiplex PCR-based assay. Infect. Immun. 68: 1587–1599.