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SEROTYPE AND BIOTYPE PREVALENCE OF AVIAN PATHOGENIC ECHERICHIA COLI IN ALBANIAN POULTRY INDUSTRY

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Abstract

A total of 104*Escherichia coli* strains isolated from affected and apparently different poultry species, breaded within the Albanian territory were serotyped and biotyped. Although *Echerichia coli* is normally present in the microflora of the poultry intestinal tract, certain subsets of *extraintestinalE.coli* strains termed as *avian pathogenic Escherichia coli* posses specific virulence factors that in previous studies have been associated with the avian colisepticaemia. Eight different serotypes were identified, where O86 resulted the most prevalent serogroup 15, 4%. Eleven biotypes were identified by the fermentation of five different sugar mediums by *E.coli* isolates. The most prevalent biotype was B31 (28, 84%). Various serobiotypes were identified, 98, 8% of *E.coli* strains were positive to rhamnosis fermentation. This study objective was the comparison of the main characteristics of Avian Pathogenic
Escherichia Coli isolated strains with the Avian Faecal Coli, in order to ascertain whether Avian Pathogenic Escherichia Coli or Avian Faecal Escherichia Coli are distinct. The results obtained from the two groups reveal for major and fundamental difference which is reflected clinically.

Key words: Colisepticaemia, *E.coli*, APEC, Biotype, Serogouping

Introduction

Collibacillosis is one of the principal causes of morbidity and mortality in avian species and is responsible for severe economic losses in the poultry industry (Dho-Moulin &Fairbrother, 1999). The disease in poultry is due to several virulence factors, which constitute various groups of similar *E.coli* strains differenced by the specific pathotype (Kaper et al., 2004). Since E.coli infections in poultry may take any clinical form including colisepticaemia, peritonitis, cellulitis, salpingitis, synovitis, omphalitis, air sacculitis and coligranuloma (Barnes et al., 2003), it seems reasonable that multiple pathotypes of "disease-causing" avian *E.coli* might exist. However, these different forms of the disease are similar in their extraintestinal tropism. Previous studies have demonstrated that APEC possess virulence properties witch promote the bacterial colonization and/or tissue invasion, subsequently leading to the colibacillosis development (Delicato et al., 2003; Dho-Moulin and Fairbrother 1999; Dozosis et al., 1992; Foley et al., 2002 and La Ragione et al., 2002). Never the less AFEC (Avian Fecal *Escherichia Coli*) strains are considered as normal part of intestinal microflora, certain *E.coli* strains are able to cause infection, and this ability due to the specific virulence genes designs them as APEC (Avian Pathogenic *Echerichia Coli*) (Dho-Moulin and Fairbrother, 1999). Several studies have revealed that generally many avian septicaemic*E.coli* are grouped into a limited number of O serotypes as: O1, O2, O15, O35 and O78 (Cloud et al., 1985; Dozosis et al., 1992; Gross, 1991; White et al., 1998). During recent studies several isolates from affected poultry specimens have mainly belonged to O18, O81, O115, O116

and O132, which merges a signal of emergency for new pathogenic serotypes (Barnes et al., 2003). However, other studies have shown that a wide antigenic diversity exists among avian pathogenic *E.coli* strains (Allan et al., 1993; White et al., 1993) and the involvement of a particular O serotype in the infection appears to vary by the geographic region. Surveys on the virulence abilities of APEC strains have demonstrated genetic semblances with human extraintestinal and uropathogenic*E.coli* strains (Kylie et al., 2005; Sorsa et al., 2001). Furthermore, several studies have shown that some APEC strains could belong to the same clones of human extraintestinal pathogenic *E.coli* (Achtman et al., 1986; White et al., 1993; White et al., 1990).
In this paper, we report the results of an epidemiological study on the circulating *E.coli* strains within the Albanian territory; comparing 74 *E.coli* isolated from colibacillosis affected poultry with 30 AFEC strains. For this aim, a total of 104 E.coli strains, from apparently healthy birds and colibacillosis affected ones were serotyped. More over the belonging biotype has been assessed.

Materials and methods

Bacterial strains

A total of 104 *E.coli* strains, 74 from clinically confirmed cases of poultry colibacillosis and 30 from visceral organs of slaughtered apparently healthy birds were tested. The sampling was performed in broth rural and intensive breeding flocks, in different random geographical areas within the Albanian territory. Also different ages and poultry specimens were involved in the study.

Table 1. Avian E.coli isolates sampling distribution according to clinical signs, the breeding type and avian species

Avian species	Apparently healthy birds		Colibacillosis affected birds		Total of *E.coli* isolated strains
	Intensive Breeding	Rural Breeding	Intensive breeding	Rural Breeding	
Broilers	6	18	14	22	60
Layers	0	6	28	0	34
Turkeys	0	0	0	10	10
Total	6	24	42	32	104

Samples of fresh visceral organs from different poultry specimens with colibacillosis lesions (liver and spleen) and from apparently healthy birds were cultured on MacConkey agar (OXOID) and incubated at 37 ^{o} C for 24h. The biochemical identification was performed using the API-20E system (Bio-MEIREUX). All the *E.coli* isolates were stored at -80 ^{o} C in Brucella broth (OXOID) with 20% of grycerol till use.

Fermentation of lactose

Test and control organisms were plated on MacConkey agar (OXOID) and incubated overnight at 37 ^{o} C. Isolates were considered positive to lactose fermentation if pink colonies were observed (Forbes et al., 1998).

O-antigen serogrouping

The serotype of each isolate was determined using a slide agglutination technique on a panel of 40 different anti-O-sera, according to the laboratory methods and international literature of clinical diagnosis for poultry, rabbits and livestock (Blanco et al., 1998). A panel of 40 different specific antisera (O1, O2, O4, O6, O8, O9, O10, O11, O15, O18, O20, O21, O22, O26, O45, O49, O64, O73, O75, O78, O83, O85, O86, O88, O92, O101, O103, O109, O111, O128, O132, O138, O139, O141, O147, O149, O153, O157). A suspension of each isolate was diluted in 1 ml PBS and heated for 1h at 100 ^{o}C. A 5µl sample of each was mixed with quantity of an O-serotype antiserum. Agglutination on a glass slide within 1 min determined the respective serotype.

Biotyping

Fermentation activity was tested on phenol red agar base, supplemented with 1% of each selected carbohydrate, in square Integrid Petri dishes (according to Difco Laboratories, Detroit, Mitch.). *E.coli*strains were inoculated as spots on the medium. The results were read after 24 and 48h of incubation at 37 ^{o}C. To assess the belonging biotype we have used the simple biotyping scheme of Camguilhem& Milton, 1989. The score number was assigned to every positive fermentation reaction as follows: D-raffinose (test score 4), L-ramnose (test score 16), dulcitol (test score 2), sucrose (test score 8) and sorbose (test score 1).

Results and discussion

Lactose fermentation - The abilities of APEC and AFEC strains to ferment lactose were determined by standard methods. Of the APEC isolates, 91% were positive to lactose fermentation; whereas 99% of AFEC isolates were lactose positive.

Serotyping – The serotyping was performed on a total of 104 avian *Escherichia coli* strains, from which only 44 isolates were typeable (42, 3%). Among the revealed serotypes were O2, O8, O15, O73, O86, O102, O115 and O139. The most frequent serotype was O86 (15, 4%) and the less prevalent were O15, O101 and O115 (1, 9% each). In medium level 4 other serotypes were detected: O2 (5, 8%), O8 (5, 8%), O73 (5, 8%) and O139 (3, 8%). The serotype O86 was more frequent in colibacillosis affected birds (87, 5%) comparing with 12, 5% of AFEC strains.

The serotypes O8, O15, O101 and O139 were only revealed from colibacillosis affected poultry, as the O115 belonged to isolates originated from apparently subjects. 60 of tested strains (57, 7%) did not belong to any serotype of the panel test, *as they were typeable with more than one specific serotype or not serotypeable at all.*

Table 2. Relationship between serotypes of E.coli strains isolated from affected and apparently healthy poultry.

E.coli isolates from colibacillosis affectedpoultry				*E.coli* isolates from apparently healthy poultry			
Serogroup	Broilers	Layers	Turkeys	Broilers	Layers	Turkeys	Total (%)
O2	0	4	0	0	2	0	6 (5, 8%)
	2	2	2	0	0	0	6 (5, 8%)
O8	0	0	2	0	0	0	2 (1, 9%)
	4	0	0	2	0	0	6 (5, 8%)
O15	10	2	2	2	0	0	16 (15, 8%)
	0	0	2	0	0	0	2 (1, 9%)
O73	0	0	0	2	0	0	2 (1, 9%)
	2	2	0	0	0	0	4 (3, 8%)
O86	18	18	2	18	4	0	60 (57, 7%)
O101							
O115							
O139							
NT							
Total	36	28	10	24	6	0	104 (100%)

Biotyping – A number of 11 different biotypes was revealed (B0, B16, B17, B18, B21, B22, B23, B28, B29, B30 and B31). 102 *E.coli* strains (98, 8%) were positive to ramnose fermentation (those with test score 16 and up). The majority (82, 67% or 86 of 104 strains) of avian *E.coli* strains were assigned to only four biotypes (B16, B28, B30 and B31). Most of the strains belonged respectively to biotype B30 (25, 0%) and B31 (28, 84%). These biotypes were more prevalent on the isolated originating from infected subjects (B31) or/ and exclusively detected in APEC strains (B30). The relevant results are presented in table number 5 and the co-relation between O-serotypes and biotypes is listed in the table number 4.

Table 3. Relationship between biotypes of E.coli strains isolated from isolated from affected and apparently healthy poultry.

E. coli isolates from colibacillosis affected poultry				*E.coli* isolates from apparently healthy poultry			
Biotype	Broilers	Layers	Turkeys	Broilers	Layers	Turkeys	Total (%)
B0	0	0	2	0	0	0	2 (1, 928%)
	6	4	0	10	0	0	20 (19,
B16	4	0	0	0	0	0	23%)
	0	0	0	2	0	0	4 (3, 84%)
B17	0	0	0	0	2	0	2 (1, 92%)
	0	0	0	0	2	0	2 (1, 92%)
B18	0	2	0	0	0	0	2 (1, 92%)
	2	2	4	2	0	0	2 (1, 92%)
B21	2	2	0	0	0	0	10 (9, 61%)
	12	12	2	0	0	0	4 (3, 84%)
B22	10	6	2	10	2	0	26 (25%)

B23 B28 B29 B30 B31					6	0	30 (28, 84%)
Total	36	28	10	24	6	0	104 (100%)

O: B – serobiotypes. The most prevalent serobiotypes detected among the 104 *E.coli* isolates, respectively to frequency were: O86: B31 (3 strains); O8: B16 (2 strains); O86: B16 (2 strains). O86: B28 (2 strains) and O139: B30 (2 strains)

Table4. Relationship between serogroups and biotypes of E.coli strains isolated from isolated from affected and apparently healthy poultry.

Serotypes	Biotypes
O2	B21 (1 strain) B23 (1 strain) B31 (1 strain)
O8	B29 (1 strain) B30 (2 strains)
O15	B28 (1 strain)
O73	B17 (1 strain) B18 (1 strain) B31 (1 strain)
O86	B16 (2 strains) B17 (1 strain) B28 (2 strains) B31 (3 strains)
O101	B0 (1 strain)
O115	B16 (1 strain)
O139	B30 (2 strains)
Non Typeable	B16 (7 strains) B22 (1 strain) B28 (2 strains) B29 (1 strain) B30 (9 strains) B31 (10 strains)

In Albania, as in other developing countries colibacillosis occurs frequently in both breeding types of the poultry industry. However the mortality varies from low to very high, depending on the characteristics of strains involved in the infection. Strains of low pathogenicity mostly cause problems in poultry farms of poor management and hygiene, which canbe easily controlled by several hygienic measures and/or antibiotic treatment. On the other hand recently, antimicrobial treatment failures are very commune due to the high pathogenicity of *E.coli* strains or to the antimicrobial resistance. The serogroups identified on 104 *Escherichia coli* isolates were O86 (15, 4%), O2 (5, 8%), O8 (5, 8%), O73 (5, 8%), O139 (3, 8%), O15 (1,9%), O101 (1, 9%) and O115 (1, 9%).

This result is very important for better understanding the profile of *E.coli* strains circulating now days in Albania. Also the serogroups identified exclusively in APEC strains, which are considered virulent were: O8, O15, O101 and O139. This result is consistent with other reports where these serogroups are commonly associated with avian colibacillosis and

confirms their role of potential pathogens in the extraintestinal infections of poultry (Dho-Moulin and Fairbrother, 1999; Ewers et al., 2004; Giovanardi et al., 2005 and La Ragione et al., 2002).

Very interesting was also the relationship of O86 with the respective Biotype, in *E.coli* strains. The biotype B31 was the most prevalent in the strains serogrouped in O86, followed by minor percentages of B16 and B28. These biotypes as presented in the table number 3 are mainly frequent in APEC strains. This induces the hypothesis that the O86 serogroup identified in an avian *E.coli* strain may be a sign of virulent abilities.

No previous study in Albania is performed before on the biotyping of avian pathogjenic and fecal *Escherichia coli*. According to the now known pathogenicity of several serotypes on the poultry health and their relationship with the identified biotypes, we can make sense that B30 and B31 are the most prevalent biotypes in acolibacillosis infection outbreak. But a conclusion on specific biotypes among pathogenic or fecal *E.coli* isolates cannot be framed as a large percentage of the identified *E.coli* biotypes coincide with the non typeable strains.

The presence of a large percentage of untypeable strains has been a common characteristic of many previous studies about the phenotypic characterization of *E.coli* strains, depending on the geographical region. A reason for 57, 7% of untypeable strains may be that the serotyping assay was performed in Italy, where the panel of 40 anti-O-sera could not possibly include the specific serogroups circulating in Albania.

Conclusions

In conclusion, our results support that a wide serological diversity among avian pathogenic and fecal *Escherichia coli* strains exists, because of the opportunistic nature of this bacterial genius. Predisposing factors (mycoplasmal or viral infections, environmental conditions and poultry breeding type) could be responsible for this wide serological diversity. These data are the first report on the serological and biotyping profiles of avian *Escherichia coli* circulating in the now days, in Albania territory, and they provide a database on what is different and similar with other countries.

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References

Achtman, M., Heuzenroeder, M., Kusecek, B., Ochman, H., Caugant, D., Selander, R.K., Vaisanen-Rhen, V., Korhonen, T.K., Stuart, S., and Orskov, F. (1986).Clonal analysis of *Escherichia coli* O2:K1 isolated from diseased humans and animals. Infect. Immun. 51(1): 268-276.

Allan, B.J., Van den Hurk, J.V., Potter, A.A., (1993). Characterization of Escherichia coli isolated from cases of avian colibacillosis. Can. J. Vet. Res., vol. 57, pg 146 – 151.

Barnes, H.J., Vaillancourt, J.P., Grass, W.B., (2003).Collibacillosis, Saif Y.M., Diseases of Poultry, Calneck et al., 11thedt, Iowa State University Press, Ames, 631 – 652.

Blanco, J. E., Blanco, M., Mora, A., Jansen, W. H., Garcia, V., Vazquez, M L., Blanco, J., (1998).Serotypes of *Escherichia coli* isolated from septicaemic chickens in Galicia (northwest Spain). *Vet. Microbiol.*61, 229 – 235.

Camgiulhem, R., and Milton, A., (1989). Biotypes and O serogroups of *Escherichia coli* involved in intestinal infections of weaned rabbits: clues to diagnosis of pathogenic strains. J. Clin. Microbiol. 27: 743-747.

Cloud, S.S., Rosenberger, J.K., Fries, P.A., Wilson, R.A. and Odor, E.M., (1985). In vitro and in vivo characterization of avian *Esherichia coli.* I. Serotypes, metabolic activity and antibiotic sensitivity. *Avian Dis.*, 29: 192-196.

Delicato, E.R., de Brito, B.G., Gaziri, L.C., Vidotto, M.C.(2003). Virulence-associated genes in *Escherichia coli* isolates from poultry with colibacillosis. Vet. Microbiol. 94, 97–103.

Dho-Moulin M., Fairbrother J.M., (1999). Avian pathogenic *Escherichia coli*. *Vet. Res.*, 30, 299-316.

Dozois, C.M., Fairbrother, J.M., Harel, J., Bosse, M., (1992). *pap*- and *pil*-related DNA sequences and other virulence determinants associated with *Escherichia coli* isolated from septicemic chickens and turkeys. Infect. Immun. 60, 2648–2656.

Dozois, C.M., Dho-Moulin, M., Bree, A., Fairbrother, J.M., Desautels, C., Curtiss III, R.(2000). Relationship between the *tsh* autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the *tsh* genetic region. Infect. Immun. 68, 4145–4154.

Ewers, C., JanBen, T., KieBling, S., Philipp, H.C., Wieler, L.H.(2004). Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. Vet. Microbiol. 104, 91–101.

Foley, S.L., Horne, S.M., Giddings, C.W., Robinson, M., Nolan, L.K.(2002). Iss from a virulent avian *Escherichia coli*. Avian Dis. 44, 185–191.

Giovanardi, D., Campanari, E., et al., (2005). Avian pthogenic Eschericia coli transmission from broiler breeders to their progeny in an integrated poultry production chain, Avian Pathology, 34, pg 313 – 318.

Gross, W.B., (1991). Colibacillosis. In: M.S. Hofstad, H.J. Barnes, B.W. Calnek, C.F. Beard, W.M. Reid and H.W. Yoder, Jr. (Editors), Diseases of Poultry. Iowa State University Press, Ames, Iowa, pp: 138-144.

Kaper, J. B., Nataro, J. P., Mobley, H. L., (2004). Pathogenic Escherichia coli. Nat. Rev. Microbiol. 2, 123-140.

La Ragione, R.M., Woodward, M.J., (2002). Virulence factors of *Escherichia coli* serotypes associated with avian colisepticaemia. Res. Vet. Sci. 73, 27–35.

Moulin-Schouleur, Maryvonne, et al., (2006). Common virulence factors and genetic relationship between O18:K1:H7 Escherichia coli isolates of human and avian origin. J. Clin. Microbiol. 44: 3484 – 3492.

Sorsa, L. J., Dufke, S., Heesemann, J., Schubert, S., (2003). Carachterization of an iriBCDEN gene cluster on a trasmissible plasmid of uropathogenic Escherichia coli: evidence for horizontal transfer of a chromosomal virulence factor. Infect. Immun. 71, 3285-3293.