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BIOLOGICAL CHARACTERISTICS AND PATHOGENICITY OF AVIAN *ESCHERICHIA COLI* STRAINS FROM ALBANIAN POULTRY FLOCKS

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Abstract

A total of 129 pathogenic Escherichia coli strains (APEC) isolated from hens and broilers suffering from colisepticaemia and ovaritis were studied regarding their biological and pathogenic characteristics such as serogroups and virulence associated genes. For comparison were studied also other 100 poultry fecal E.coli strains originated from apparently healthy birds. Serotyping demonstrated that most of E.coli strains were untypable in both colibacillosis clinic division groups (62% for and 34%, whereas in 129 of E. coli strains the searched eight virulence genes for their presence showed relationship with colibacillosis infection outbreaks in poultry. Serotyping identified a very wide variety of serotypes according to APEC and AFEC strains. Serotypes most often associated with the presence of clinical signs resulted O86 (8, 75%), O2 (4, 86%), O8 (6, 77%), O15 (3, 88%), O139 (2, 92%) ; O157 (2.92%) 78 O (1, 94%), while those with apparently healthy birds: O8 (11, 53%), O157 (7, 69%), O73 (3, 85 %), O86 (3, 85%), O115 (3, 85%) and O2 (3, 75%). The lack of virulence factors in APEC strains resulted 18, 05%, while in AFEC strains 81, 95%. This study identified significant differences of virulence factors among strains isolated from lesions, compared to those from apparently healthy subjects. Anyway the detection of virulence genes present in serotypes O15, O86, O73, O101, O147, O157, brings a wider variety of APEC in serogroups classification. These data obtained from genetic characterization of avian E.coli strains constitute the first report in Albania, for colibacillosis infection outbreak in poultry flocks. The presence, appearance and distribution of virulence genes in poultry flocks, provides basic information for the control and eradication of the colibacillosis infection. Application of molecular biology methods to further knowledge of the serotyping data now is a time requirement for the prevention and eradication of avian colibacillosis or other bacterial poultry infections.

Key words: *Avian Pathogenic Escherichia coli, Serotyping, Genotyping, colibacillosis, virulence genes*

INTRODUCTION

Colibacillosis caused by Avian Pathogenic *Escherichia coli* strains, is the main cause of economic losses in poultry industry, worldwide [1]. This acute infection is clinically localized and systemic, with variety of lesions in organs viscera.

Currently, pathogenic *Escherichia coli* infections are more frequently encountered in intensive poultry breeding flocks. Colibacillosis presence and its outbreaks are considered as an important indicator of the level of poultry productivity and growth.

Initial studies on avian *E. coli* strains have shown that O1, O2 and O78 serotypes, are mostly associated with colibacillosis outbreaks [3]. While half of the strains examined in many studies are not fully elucidated to settle in classical serogrouping classification, making these strains untypeable. In now days serotyping does not constitute a basis for *E. coli* diagnosis and identification, especially it does not specify the fact if a serotype expresses the virulence of the strain, but this test is important for epidemiological studies.

Clear identification and differentiation of Avian *Escherichia coli* with opportunistic *E. coli* remains a lack for the veterinarian research science of laboratory diagnosis. For this reason the serotyping of 129 avian *E.coli* isolates would help to situate a database of epidemiological evidences of the actual situation in the poultry industry about the circulation *E.coli* strains in Albania.

MATERIAL AND METHOD

On 129 avian *E.coli* isolates was conducted the serotipization using a standardized panel of 40 monoclonal specific antiserums against the somatic O antigen (O1, O2, O3, O4, O6, O8, O9, O10, O11, O15, O18, O20, O21, O22, O26, O45, O49, O64, O68, O73, O75, O78, O83, O85, O86, O88, O92, O101, O103, O109, O111, O115, O128, O132, O138, O139, O141, O147, O153 and O157){2}.
Serotyping was performed at Experimental Zooprophilactic Institute of Brescia, Italy, which uses the O anti serums cited above, in accordance with *E.coli* strains circulating in poultry in the area concerned.

RESULTS AND DISCUSSIONS

The serotipization of 129 *E.coli* strains, isolated from colibacillosis affected and aparently healthy birds allowed the characterization of only 31, 21% of them. Untypeable strains resulted 81 or 62, 79% of them (Tab. No. 1).

Table 1: Serogrups identified in APEC and AFEC strains (num = 129*)*

Untyped Serotyped	81 48[a]	62,79[b] 37,21
O2	6	4,65
O8	10	7,75
O15	4	3,10
O73	3	2,33
O78	2	1,55
O86	10	7,75
O88	2	1,55
O101	1	0,77
O115	1	0,77
O139	3	2,33
O147	1	0,77
O157	5	3,89

Serogrouping identified 12 different serotypes with the following percentages: O8 (7.75%) O86 (7.75%) O2 (4.65%) O157 (3.89%); O15 (3.10 %), O73 (2.33%), O139 (2.33%), O78 (1, 55%), O88 (1, 55%), O101 (0, 77%), O115 (0, 77%) and O147 (0, 77%).
More than half of *E.coli* strains (62, 79%) were not serogrouped because they failed to react with the standard antisera panel [2]. Thus, previous data on similar levels of untypeable levels of *E.coli* strains, comes up the discussion whether or not serogrouping is an efficient method of characterizing avian *E.coli*.
Shortcomings of this method relate to the existence of autoagglutinating strains or cross reacting ones with more than one O antiserum, all this depending on specific geographic regions [6].
Results of previous epidemiological research, in many countries indicate that only 15% of strains belonging serogroups O1, O2, O35, O36 and O78, or are associated with *E.coli* infections, the rest is part of unknown serogrups (untyped) or represent new serotypes. This should be taken as a signal for the presence of new pathogenic serotypes not yet studied {4}. If the untyped strains resulted 62, 79%, this does not mean that they should be considered as non-pathogenic, as many of them contained in bacterial genome responsible for virulence genes and originated colibacillosis affected birds.
Serotyping identified a very wide variety of serotypes according to APEC and AFEC strains. Serotypes most often associated with the presence of clinical signs resulted O86 (8, 75%), O2 (4, 86%), O8 (6, 77%), O15 (3, 88%), O139 (2, 92%) ; O157 (2.92%) 78 O (1, 94%), while those with apparently healthy birds: O8 (11, 53%), O157 (7, 69%), O73 (3, 85 %), O86 (3, 85%), O115 (3, 85%) and O2 (3, 75%).
Serotypes O2, O8, O73, O86 and O157 were present as in APEC, so in AFEC. While serotype's O15, O78, O88, O101, O139 and O147 presence resulted associated only with APEC. Given that serotypes: O2, O8, O15, O78, O115 and O139, were related to APEC strains, while AFEC had the presence of O2, O8, O157 serotypes, we can say that serotyping serves as a method of classification and not to define the pathogenicity of *E.coli* strains.
Our results support the fact of the existence of the wide serological diversity among avian *E.coli* isolates, may come as a result of the opportunistic nature of most infections.
Predisposing factors (Mycoplasmosis or viral infections and environmental factors) may be responsible for this diversity.

Table 2: The distribution of serotypes according to APEC and AFEC strains

Serotypes	APEC	%	AFEC	%
O2	**5**	**4,86**	**1**	**3,85**
O8	**7**	**6,77**	**3**	**11,53**
O15	**4**	**3,88**	**0**	**0,00**
O73	**2**	**1,94**	**1**	**3,85**
O78	**2**	**1,94**	**0**	**0,00**
O86	**9**	**8,75**	**1**	**3,85**
O88	**2**	**1,94**	**0**	**0,00**
O101	**1**	**0,97**	**0**	**0,00**
O115	**0**	**0,00**	**1**	**3,85**
O139	**3**	**2,92**	**0**	**0,00**
O147	**1**	**0,97**	**0**	**0,00**
O157	**3**	**2,92**	**2**	**7,69**
NT	**64**	**62,14**	**17**	**65,38**
TOTAL	103	100,00	26	100,00

Since in this study was revealed a large number of serotypes, previous studies recommend that the use of an effective vaccine which should summarize in a wide range avian *E.coli* serotypes [5]. Serotyping is not the only method of defining the pathogenic behaviour of *E.coli,* because it not always coincide with a wider genetic diversity among strains of a serotype [3; 7; 8]. For this reason in this research was studied the co-relation between the identified the serotypes with the presence of virulence associated genes as presented in table no.3.

Table 3: The co-relation of virulence associated genes with the identified serotypes on 129 E. coli strains

	8 virulence associated genes							
Serotypes	astA1%	iss%	Irp2%	iucD %	papC %	tsh %	Vat%	CvaA/B %
O2 (no = 6)	50,00	66,66	50,00	66,66	33,33	66,66	33,33	33,33
O8 (no = 10)	30,00	50,00	30,00	40,00	20,00	20,0	20,00	20,00
O15 (no = 4)	25,00	75,00	75,00	25,00	0,00	0,00	0,00	25,00
O78 (no = 2)	0,00	100,00	100,00	100,00	50,00	0,00	0,00	0,00
O86 (no = 10)	20,00	20,00	10,00	10,00	0,00	0,00	0,00	0,00
O73 (no = 3)	66,66	66,66	0,00	0,00	0,00	0,00	0,00	0,00
O101 (no = 1)	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00
O115 (no = 1)	0,00	100,00	100,00	100,00	0,00	100,00	0,00	100,00
O139 (no = 3)	66,66	66,66	66,66	66,66	66,66	0,00	66,66	66,66
O147 (no = 1)	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00
O157 (no = 5)	0,00	20,00	0,00	20,00	0,00	0,00	0,00	0,00
O88 (no = 2)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NT (no = 81)	23,40	53,09	35,8	40,74	20,99	24,69	8,64	8,65

The co-relation between virulence associated genes and the identified serotypes proides a clear picture of the genetic diversity within certain serotype or further more a serogroup. As shown on the table no. 3 serotypes O2, O8 and O78 which are frequently related with avian colibacillosis infection contain virulence factors in moderate leveles.

Serotypes O139 and O115, although bear in a significant degree of virulence factors, are not reported in previous studies [2] as implicated in avian colibacillosis. This result may be a reflection of regional differences related to the prevalence of different serogroups in different geographical areas. The presence of virulence genes present in serotypes O15, O86, O73, O101, O147, O157, except those known as pathogens,

shows a wider variation of APEC in serogrouping classification.

CONCLUSIONS

Serogrouping identified 12 different *E.coli* serotypes. The untyped strains resulted at a level of 62, 79%. Serotypes: O15, O73, O86, O101, O115, O139 and O147 represent new entrance in colibacillosis patogenesis. This should be taken as a signal for the presence of new pathogenic serotypes not yet elucidated.

Serotyping identified a very wide variety of serotypes according to APEC and AFEC strains. Serotypes O139 and O115 carried significant levels of virulence factors, although they have not been identified previously as avian colibacillosis implicated serotypes. This result indicated that the prevalence of clonal *E.coli* groups depends on the specificity of a geographical area.

This study results suggest that serotypes known as pathogens that have correlation with virulence factors and are closely associated with the colibacillosis outbreak in poultry. This conclusion applies those untypeable strains too, which in their genome contain important virulence genes.

The application of molecular biology methods to further knowledge of the serotyping data now is a time requirement for the prevention and possible eradication of avian colibacillosis or other bacterial poultry infections.

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