VIRULENCE-ASSOCIATED GENES *IN AVIAN PATHOGENIC ESCHERICHIA COLI* OF TURKEY

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Abstract
50 *Escherichia coli* (*APEC-Avian Pathogenic Escherichia coli*) strains and 15 *E. coli* (*AFEC-Avian Faecal Escherichia coli*) from turkeys affected by colibacillosis and from healthy turkeys were tested for the presence of eight different virulence-associated genes. Besides, *APEC* were serotyped. O78 has been the most detected serotyped. The presence of the tested virulence genes was prevalently related to the *APEC* isolates. With reference to serogroup, all the tested O78 resulted *iss* and *irp2* positive. Besides, *tsh* e *cva/cvi* were respectively present in 88.9 and 83.3 % of O78. Nevertheless, the finding of a not typeable strains equipped with all the eight tested virulence genes among the *APEC* isolates suggest the importance of a careful and complete characterisation of the isolate to evaluate the real potential pathogenic attitude of the bacterium.

Key words: *Escherichia coli*, turkeys, serotyping, virulence genes

GENI DI VIRULENZA IN *AVIAN PATHOGENIC ESCHERICHIA COLI* NEL TACCHINO

Riassunto
In questa ricerca, 50 stipiti di *E. coli* isolati da tacchini affetti da colibacillosi (*APEC)* e 15 *E. coli* provenienti dal contenuto intestinale di soggetti sani (*AFEC*) sono stati caratterizzati e sottoposti alla ricerca di 8 differenti geni di virulenza. Gli stipiti *APEC* sono inoltre stati sierotipizzati al fine di evidenziare i sierotipi più frequentemente associati alla malattia. Tra questi, O78 è risultato il sierotipo di gran lunga prevalente. I geni di patogenicità ricercati sono risultati fortemente associati agli stipiti patogeni rispetto a *E. coli* di origine fecale. Considerando il sierotipo di appartenenza, la totalità di O78 testati presentava i geni legati ai sistemi di acquisizione del ferro ed una elevata percentuale risultava *tsh* e *cva/cvi* positiva, confermando il potenziale ruolo di tali geni nella patogenicità di tali sierotipi. Il riscontro tuttavia di uno stipite non tipizzabile sierologicamente e munito di tutti gli 8 geni di virulenza ricercati pone l'attenzione sull'importanza di effettuare una completa e accurata caratterizzazione dell'isolato per poterne valutare l'effettivo potenziale patogeno.

Parole chiave: *Escherichia coli*, tacchini, sierotipizzazione, geni di virulenza

Introduction

Colibacillosis infections are cause of important economic losses in turkey industry. These infections are often characterised by respiratory lesions, in particular airsacculitis associated with pericarditis, perihepatitis and peritonitis. Environmental factors or viral infections may influence the outcome of colibacillosis. Nevertheless, avian pathogenic *Escherichia coli* (*APEC*) may play a role as single aetiological agent. Moreover, *E. coli* superinfection may increase the pathogenic attitude of mycoplasma or *Chlamydophila psittaci* in turkey (Van Loock *et al.* 2006).

The presence of virulence-associated genes seems to be linked to the pathogenic attitude of the bacteria. Nevertheless, this association is still uncertain and their exact role is yet unclear.

In this study, the presence of eight different virulence-associated genes has been investigated in *E. coli* from turkeys affected by colibacillosis. To this aim, 50 *E. coli* (APEC-Avian Pathogenic Escherichia coli) were collected, serotyped and tested for the presence of the putative virulence genes. Besides, 15 E. coli (*AFEC-Avian Faecal Escherichia coli*) were collected from the gut of healthy turkeys to compare and better interpret the obtained results.

Materials and methods

50 *Escherichia coli* (*APEC-Avian Pathogenic Escherichia coli*) strains and 15 *E. coli* (*AFEC-Avian Faecal Escherichia coli*) were respectively collected from turkeys affected by colibacillosis and from healthy turkeys coming from different intensive farms of Italy. Each strain was cultured on MacConkey agar (OXOID) and incubated at 37 °C for 24h. Every compatible colony was isolated on Trypticase Soy Agar (TSA) (OXOID) and incubated at 37 °C for 24h. The biochemical identification was carried out using the API-20E method (Bio-MERIEUX). All *E. coli* strains were stored at -20 °C in Brucella broth (OXOID) with glycerine (20%) before the execution of the characterisation test.

Serotyping. The *APEC isolates* were serotyped. Serotyping was carried out using monospecific antisera towards 40 different somatic O antigens (O1, O2, 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, O149, O153, O157) in U bottom polystyrene microtitre plates incubated for 24 hours at 37 °C in a moist box (Blanco and Blanco, 1993).

Genotyping. The genetic characterisation was performed on *APEC* and *AFEC* isolates using a Multiplex-PCR (Polymerase Chain Reaction) according to Ewers *et al.* (2005). *E. coli* were tested for the presence of enteroaggregative toxin (*astA*), increased serum survival protein (*iss*), iron-repressible protein (*irp2*), aerobactin (*iucD*), P-fimbriae (*papC*), temperature-sensitive hemagglutinin (*tsh*), vacuolating autotransporter toxin (*vat*), colicin V plasmid operon genes (*cva/cvi*).

Results and discussion

A relevant number among the APEC isolates were typeable using somatic O antisera. 14 different serogroups were identified (table 1). In order of frequency, the most de-

tected serogroups associated to the disease have been O78 (36%), O8, O73 (6%), O2, O9, O20, O139, O141 (4%).
The detection of the virulence-associated genes was most related to the *APEC* isolates, in particular for *iss*, *iucD*, *cva/cvi* (table 2). Moreover, *irp2*, *tsh* and *vat* have been exclusively found in the septicaemic strains.
The greater association observed of the putative virulence genes to the septicaemic isolates may increase the potential pathogenic attitude of the bacteria by different mechanisms. For example, *irp2* and *iucD* encode for two different systems of iron acquisition as yersiniabactin and aerobactin, respectively, and may increase the survival of the bacterium in the host in conditions of poor density of iron. *tsh*, which encodes for a temperature sensitive hemagglutinin, seems to be important in the early stages of the colonisation of the respiratory tract and for the invasion of the bloodstream (Ewers *et al.* 2003). *vat* encodes for a vacuolating autotransporter toxin while *papC* for the P-fimbriae which allows E. coli to adhere to the tissue and protect the bacterium from the inflammation cells activity .*cva/cvi* is indicative of the presence of *col V*-plasmid which may serve as vector of several putative virulence genes as *tsh*, *iss*, genes encoding for iron acquisition systems (Johnson *et al.* 2006) or antimicrobial resistance factors.
astA, encoding for an enteroaggregative stable toxin, is considered a virulence factor associated with diarrhoea in human and animals but was also expressed by several non-pathogenic *E. coli* in humans and animals. In our study, *astA1* was most detected among the strains from gut of healthy turkeys in respect to the *APEC* isolates (table 2).
In this research, the distribution of the virulence-associated genes in *E. coli* O78, the serogroup more frequently detected among the APEC isolates, has been of relevance. *iss* and *irp2* have been found in all O78 and tsh and *cva/cvi* were detected in the most of O78 (table 3). These genes, and in particular tsh and *cva/cvi*, were identified less frequently in *E. coli* of other serogroups or not-typeable strains. These findings confirm the importance of the presence of these genes to increase the pathogenic attitude of the bacterium. In fact, O78 notoriously represent the most associated serogroup to the disease in poultry (Barnes *et al.* 2003).
vat was never found in O78 (table 3). In previous observations, this gene was absent or sporadically detected in O78 while frequently associated with O2 (Vandekerchove et al. 2005). Likewise, the O2 strains detected in this study were *vat*-positive. At present, we are not able to explain the reason of this apparent correlation between *vat* and O2 serogroup. Finally, we point out the finding of a not-typeble strain among the APEC isolates equipped with all the eight tested virulence genes.

Conclusion

In this study, a relevant correlation between the virulence-associated genes investigated and the *E. coli* strains from affected turkeys was observed. The virulence genes more frequently detected among the *APEC* strains have been *iss*, *iucD*, *cva/cvi*. *Iss* and *iucD* were present in all tested O78, while tsh and cva/cvi were of high relevance in O78 in respect to other serogroups. These observation are, in our opinion, of relevance and prove the importance of these virulence-associated genes, considering that O78 has been the most detected serogroup among the *APEC* isolates and is notoriously related to the disease. The finding of a not-typeable APEC strain equipped with all the eight tested genes confirms the importance of characterisation in depth to define the potential pathogenic attitude of the isolate.

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References
Barnes H.J., Vaillancourt J., Gross W.B. Colibacillosis. 2003. In Disease of Poultry. 11th Edition. Editor in Chief Saif Y. M. Associated Editors Barnes H. J., Glisson J. R.. Fadly A. M., McDougald L. R., Swayne D. E. 18: 631-656.
Blanco, J., Blanco, M. (1993). Escherichia coli enterotoxigenicos, necrotoxigenicos y verotoxigenicos de origin humano y bovino. Servicio de publicaciones Diputacion Provincial San Marcos, s/n. 27001 Lugo.
Ewers C., Janben T., Kiebling S., Philipp H.C., Wieler L.H., 2005. Rapid detection of virulence-associated genes in Avian Pathogenic Escherichia coli by multiplex polymerase chain reaction. Avian Dis. 49: 269-273
Ewers C., Janssen T., Wieler LH. 2003. Avian pathogenic Escherichia coli (APEC). Berl Munch Tierarztl Wochenschr. Sep-Oct; 116(9-10): 381-95
Johnson T.J., Siek K.E., Johnson S.J., Nolan L.K. 2006 DNA Sequence of a ColV Plasmid and prevalence of selected Plasmid-encoded virulence genes among avian Escherichia coli strains. Journal of bacteriology, vol. 188, n. 2, 745-758.
Van Loock M., Loots K., Van Heerden M., Vanrompay D., Goddeeris BM. 2006. Exacerbation of Chlamydophila psittaci in turkeys superinfected by Escherichia coli. Vet. Res. Nov-Dec; 37 (6): 745-55
Vandekerchove D., Vandemaele F., Adriaensen C., Zaleska M., Hernalsteen J.P., De Beats L., Butaye P., Van Immerseel F., Wattiau P., Laevens H., Mast J., Goddeeris B., Pasmans F., 2005. Virulence-associated traits in avian Escherichia coli: comparision between isolates from colibacillosis-affected and clinically healty layer flocks. Vet Microbiol 108: 75-87

Table 1. Serogroups distribution among *E.coli* from colibacillosis affected turkeys

Not typeable	10	(20)
Typeable	**40[a]**	**(80)[b]**
O1	1	(2)
O2	2	(4)
O4	1	(2)
O8	3	(6)
O9	2	(4)
O11	1	(2)
O15	1	(2)
O20	2	(4)
O21	1	(2)
O73	3	(6)
O78	18	(36)
O86	1	(2)
O139	2	(4)
O141	2	(4)

Note: [a] **Number of isolates,** [b] **% of detection**

Table 2. Virulence-associated genes in *E. coli* from colibacillosis affected and healthy turkeys

	astA1	*iss*	*irp2*	*iucD*	*papC*	*tsh*	*Vat*	*cva/cvi*
APEC (n° 50)	11[a] (22)[b]	42 (84)	27 (54)	39 (78)	8 (16)	22 (44)	8 (16)	29 (58)
AFEC (n° 15)	4 (26.7)	4 (26.7)	0 (0)	5 (33.3)	1 (6.7)	0 (0)	0 (0)	1 (6.7)

Note: [a] **Number of isolates,** [b] **% of detection**

Table 3. Virulence-associated genes in *E. coli* serogroups from turkeys affected by colibacillosis

	astA1	*iss*	*irp2*	*iucD*	*papC*	*tsh*	*Vat*	*Cva/cvi*
	2[a]		11		2	16		
O78 (n° 18)	(11.1)[b]	18 (100)	(61.1)	18 (100)	(11.1)	(88.9)	0 (0)	15 (83.3)
Other serogroups		17	13	14	3	4	6	11
(n° 22)	6 (27.3)	(77.3)	(59.1)	(63.6)	(13.6)	(18.2)	(27.3)	(50)
Not typeable								
(n° 10)	3 (30)	7 (70)	3 (30)	7 (70)	3 (30)	2 (20)	2 (20)	3 (30)

Note: [a] **Number of isolates,** [b] **% of detection**