Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

Occurrence of pathogenic and faecal *Escherichia coli* in layer hens

Antonio Camarda, Elena Circella, Donato Pennelli, Patrizia Battista, Giancarlo Di Paola, Anna Madio & Silvia Tagliabue

To cite this article: Antonio Camarda, Elena Circella, Donato Pennelli, Patrizia Battista, Giancarlo Di Paola, Anna Madio & Silvia Tagliabue (2008) Occurrence of pathogenic and faecal *Escherichia coli* in layer hens, Italian Journal of Animal Science, 7:3, 385-389, DOI: 10.4081/ijas.2008.385

To link to this article: https://doi.org/10.4081/ijas.2008.385

PROCEEDINGS OF THE SIPA 46TH CONGRESS

ITALIAN SOCIETY OF AVIAN DISEASES (SIPA), NATIONAL BRANCH OF THE WORLD VETERINARY POULTRY ASSOCIATION (WVPA)

FORLÌ (ITALY), SEPTEMBER 27-28, 2007

Edited by

ROSANNA SCIPIONI,
Editor-in-Chief

GUIDO GRILLI, ANTONIO LAVAZZA
Guest Editors

Occurrence of pathogenic and faecal *Escherichia coli* in layer hens

Antonio Camarda[1], Elena Circella[1], Donato Pennelli[2], Patrizia Battista[1], Giancarlo Di Paola[1], Anna Madio[1], Silvia Tagliabue[2]

[1]Dipartimento di Sanità e Benessere degli Animali. Università di Bari, Italy

[2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna. Brescia, Italy

Corresponding author: Prof. Antonio Camarda. Dipartimento di Sanità e Benessere degli Animali. Facoltà di Medicina Veterinaria. Università degli Studi di Bari. S.p. Casamassima Km. 3, 70010 Valenzano (BA), Italy - Tel. +39 080 4679910 - Fax: +39 080 4679910 - Email: a.camarda@veterinaria.uniba.it

ABSTRACT

A total of 117 *Escherichia coli* from colibacillosis affected (*APEC*) and clinically healthy birds (*AFEC*) were serotyped and tested for the presence of virulence genes: *iss, tsh, cva*.
A total of 54.5% *E. Coli* were typeable and 15 different serogroups were identified. The most common serogroups among *APEC* strains were O78, O2 and O128, whereas O139 was predominant in faecal strains from healthy birds.
Iss, tsh e *cva* were more frequently detected among the septicaemic *E. coli* strains. The association of virulence genes was observed. Particularly, the pathotype *iss-tsh-cva* was present in 46.5% of *APEC* strains.
Referring to serogroups, *E. coli* O78 and O2 originating from colibacillosis affected birds were always *iss-tsh-cva* positive but did not share virulence genes when they came from healthy birds.

Key words: Escherichia coli, Laying hens, Serotyping, Virulence genes, Pathotype.

RIASSUNTO

ESCHERICHIA COLI PATOGENI E COMMENSALI IN GALLINE OVAIOLE AMMALATE E SANE

Centodiciassette Escherichia coli isolati in corso di focolaio di colibacillosi (84 stipiti) e da galline ovaiole sane (33 stipiti) sono stati sierotipizzati e sottoposti a ricerca di geni di virulenza: iss, tsh, cva.
La sierotipizzazione ha permesso di caratterizzare il 56% degli stipiti e di identificare 15 differenti sierogruppi. O78, O2 e O128 risultavano i più frequenti tra E. coli isolati da soggetti ammalati, mentre agli stipiti di origine fecale era più spesso associato il sierogruppo O139.
Le percentuali di riscontro dei geni di virulenza ricercati risultavano significativamente più elevate negli stipiti provenienti dagli animali ammalati rispetto a quelli di origine fecale. Era possibile osservare l'associazione di più geni in uno stesso ceppo. In particolare, il patotipo iss-tsh-cva *si evidenziava nel 46,5% degli E. coli associati a malattia. Considerando il sierotipo di appartenenza dell' isolato, tutti gli E. coli O78 e O2 presentavano il tipo genetico* iss-tsh-cva*, quando isolati da soggetti ammalati, ma potevano presentarsi anche privi di geni di virulenza quando isolati da soggetti sani.*

Parole chiave: *Escherichia coli, Galline ovaiole, Sierotipizzazione, Geni di virulenza, Patotipo.*

Introduction

Escherichia (E.) coli infections are responsible of important economic losses in the poultry industry. Colibacillosis are widely distributed worldwide and cause systemic and localized infections. Often, colibacillosis occurs because of the action of predisposing agents, such as mycoplasmal, viral, and environmental factors. However, some *E. coli* strains seem to be more aggressive than others and some serogroups, e.g. O1, O2, O78, are more frequently associated to septicaemic clinical pictures. In poultry, the virulence mechanisms of *E. coli* infections are still few clear. However, it seems that the possession of some virulence factors may render a strain more adaptable to the host and virulent. Particularly, *iss* (*increased serum survival protein*) gene seems to improve the ability of *E. coli* to survive in the extraintestinal tissues. *Tsh* (*temperature-sensitive hemagglutinin*) encodes for the production of an heat-sensitive hemagglutinin. *Cva* encodes for the colicin production and indicates the presence of *colV* plasmid since it is a *colV* plasmid-linked factor. ColV plasmid may serve as a vector of other putative virulence traits such as e.g. antimicrobial resistance, or iron chelator systems, ect…that are likely responsible of their contribution to virulence.

In this paper, 117 *E. coli* strains selected from colibacillosis-affected and clinically healthy layer hens were serotyped and tested for the presence of *iss, tsh, cva*, to assess the possibility to correlate some specific serogroups and pathotypes to the *E. coli* strains responsible of the disease.

Material and methods

A total of 117 *Escherichia coli* strains isolated from sick birds (82 APEC-*Avian Pathogenic Escherichia coli*) and from clinically healthy birds (30 AFEC-*Avian faecal Escherichia coli*) were collected from laying hens from 23 farms of Apulia region. Each strain was cultured on MacConkey agar (OXOID) and incubated at 37°C for 24h. The compatible colonies was cultured 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 testing.

Serotyping

Serotyping was carried out using a battery of 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). U bottom polystyrene microtitre plates were used for the purpose and incubated for 24 hours at 37°C in a moist box (Blanco and Blanco, 1993).

Genotyping

E. coli were tested for the presence of *iss, tsh, cva* by multiplex PCR (Polymerase chain reaction) according to Ewers *et al.* (2005).

Results and discussion

A total of 55.6% *E. coli* were typeable and 15 different serogroups were identified. In reference to the origin of the isolate, the distribution of serotypes was variable (Table 1). In fact, O2, O78, O128 were the prominent serotypes among the septicaemic strains, with a remarkable presence of O78 and O2 in APEC (90.5, 66.7 respectively) in respect to the faecal strains.

O1, O88, O128, O149 e O153 were exclusively observed among the septicaemic

Table 1. Serogroups distribution among *E. coli* coming from colibacillosis affected and clinically healthy layer hens.

	APEC		AFEC		Total
Serogroups	N.	%	N.	%	N.
O1	1	100	0	0	1
O2	6	66.7	3	33.3	9
O6	1	50	1	50	2
O11	0	0	1	100	1
O15	0	0	2	100	2
O21	0	0	1	100	1
O78	19	90.5	2	9.5	21
O88	3	100	0	0	3
O103	1	50	1	50	2
O128	6	100	0	0	6
O139	5	55.6	4	44.4	9
O141	2	66.7	1	33.3	3
O149	2	100	0	0	2
O153	1	100	0	0	1
O157	0	0	2	100	2

E. coli. These findings seem to confirm the pathogenic attitude of some serogroups of *Escherichia coli*, *i.e.* O2 O78, O1, O88 (Altekruse *et al.*, 2002; Rodriguez-Siek *et al.*, 2005).

O11, O15, O21, O157 were exclusively found in faecal strains. O139 was the prominent serotype from healthy birds.

Virulence genes have frequently been found in APEC: *iss* 83.33%, *tsh* 46.43%, *cva* 64.29%. Less frequently, they were identified in AFEC: *iss* 48.48%, *tsh* 9.09%, *cva*12.1%. These virulence genes have been frequently associated with septicaemic strains (Delicato *et al.*, 2002; McPeake *et al.*, 2005).

The multiple presence of virulence genes in the same strain was frequently observed. It particularly occurs in the septicaemic strains. The pathotype iss/tsh/cva was frequently associated to the septicaemic strains (46.5%). Likewise, the multiple presence of virulence genes was previously observed in pathogenic *E. coli* (Giovanardi *et al.*, 2005). On the contrary, faecal strains appeared less virulent and in prevalence (51.51%) they did not have any virulence genes in this study.

With reference to the serogroup (Table 2), O2 and O78 coming from colibacillosis affected birds always had pathotype *iss-tsh-cva*. On the contrary, O78 and O2 lacking of any virulence genes were detected among the faecal strains. Likewise O11, O15, O21, generally considered as pathogen serotypes (Altekruse *et al.*, 2005; McPeake *et al.*, 2005) were exclusively found among the faecal strains in this study and did not have virulence genes. Among the untypeable strains,

Table 2. Serogroups and pathotypes distribution among *E. coli* coming from colibacillosis affected and clinically healthy layer hens.

	Serogroups	Pathotype	Virulence genes	N. APEC	N. AFEC
E. coli recovered both in colibacillosis affected and clinically healthy birds	O78	A	*iss-tsh-cva*	19	1
		D	none	0	1
	O2	A	*iss-tsh-cva*	6	2
		D	absence	0	1
	O139	A	*iss-tsh-cva*	1	0
		B	*iss-cva*	4	0
		C	*iss*	0	2
		D	none	0	2
	O141	B	*iss-cva*	1	0
		C	*iss*	1	0
		D	none	0	1
	O6	C	*iss*	1	1
	O103	C	*iss*	0	1
		D	none	1	0
APEC^{+}	O1	B	*iss-cva*	1	-
	O88	C	*iss*	2	-
		D	none	1	-
	O128	A	*iss-tsh-cva*	5	-
		C	*iss*	1	-
	O149	C	*iss*	1	-
		D	none	1	-
	O153	C	*iss*	1	-
AFEC^{++}	O11	C	*iss*	-	1
	O15	D	none	-	2
	O157	D	none	-	2
Untypeable		A	*iss-tsh-cva*	4	0
		B	*iss-cva*	9	1
		C	*iss*	9	7
		D	none	11	7

**Serogroups exclusively found in APEC strains; **Serogroups exclusively found in AFEC strains.*

the presence of virulence genes was in prevalence associated with the septicaemic *E. coli*. These results seem to prove that serotyping is an assay not effective in providing information on the real pathogenic attitude of *E. coli*. The association of molecular as-

says may be particular useful to assess this attitude.

Conclusions

The results of this study seem to confirm the pathogenic role of some specific serogroups of *Escherichia coli*. However, the application of PCR to detect virulence genes appears particularly useful in providing additional informations about the effective pathogenic attitude of *E. coli*. In fact, the possession of some virulence factors as serum survival system or ColV plasmid which encodes for several other potential virulence factors (*e.g.* bacterial antibiotic resistance, *iss, tsh* and iron chelators) may render a strain more adaptable, and improve its survival in the hosts using host's resources for replication.

The association of more than one virulence gene in the same strain may provide a particular pathogenic attitude, and may result in an increased virulence.

REFERENCES

Altekrause, S.F., Elvinger, F., DebRoy, C., Pierson, F.W., Eifert, J.D., Sriranganathan, N., 2002. Patoghenic and faecal *Escherichia coli* strains from turkeys in a commercial operation. Avian Dis. 46:562-569.

Blanco, J., Blanco, M., 1993. *Escherichia coli* enterotoxigenicos, necrotoxigenicos y verotoxigenicos de origin humano y bovino. Servicio de publicaciones Diputacion Provincial San Marcos, Lugo, Spain.

Delicato, E.R., de Brito, B.G., Konopatzki, A.P., Gaziri, L.C.J., Vidotto, M.C., 2002. Occurence of *temperature-sensitive hemagglutinin* among avian *Escherichia coli*. Avian Dis. 46:713-716.

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.

Giovanardi, D., Campanari, E., Sperati Ruffoni, L., Pesente, P., Ortali, G., Furlattini, V., 2005. Avian pathogenic *Escherichia coli* transmission from broiler breeders to their progeny in an integrated poultry production chain. Avian Pathol. 34:313-318.

McPeake, S.J.W., Smyth, J.A., Ball, H.J., 2005. Characterisation of avian pathogenic *Escherichia coli (APEC)* associated with colisepticaemia compared to faecal isolates from healthy birds. Vet. Microbiol. 110:245-253.

Rodriguez-Siek, K.E., Giddings, C.W., Doetkott, C., Jhonson, T.J., Nolan, L.K., 2005. Characterizing the *APEC* pathotype. Vet. Res. 36:241-256.