Serotypes of *E. coli* isolated from avian species in Lombardia and Emilia Romagna (North Italy)

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ABSTRACT

In this paper we report the results of n.105 *E. coli* strains serotyping, isolated during the period 2000-2004 in Lombardia and Emilia Romagna (North Italy) from avian species (poultry and turkeys), starting from cloacal swabs. The most frequently identified serogroup was O78 both in poultry and turkeys, with a large prevalence over the other detected serogroups. Remarkable was the non typeable percentage among the examined strains, datum which is in accordance with our and other authors’ previous studies.

Key Words: *E. coli*, Serotyping, Poultry, Turkey, O78.

RIASSUNTO

SIEROTIPI DI *E. COLI* ISOLATI DA SPECIE AVIARIE IN LOMBARDIA ED EMILIA ROMAGNA

Obiettivo di questo studio era procedere alla sierotipizzazione di ceppi di *E. coli* isolati da polli e tacchini, allevati nelle regioni Lombardia ed Emilia Romagna, sia in ambiente intensivo che rurale. I ceppi batterici, isolati da tamponi cloacali, sono stati saggiati con n.37 differenti antisieri O; i risultati ottenuti hanno permesso di mettere in luce una larga prevalenza del sierogruppo O78 sugli altri, in entrambe le specie testate. Degno di nota è il riscontro relativo al numero dei ceppi non tipizzati, dato che concorda con quanto già evidenziato in nostri studi precedenti e confermato anche da risultanze sperimentali di altri autori.

Parole chiave: Escherichia coli, Sierotipizzazione, Pollo, Tacchino, O 78.

Introduction

Avian pathogenic *Escherichia coli* (APEC) cause aerosacculitis, polyserositis, septicaemia and other mainly extraintestinal diseases in poultry, turkeys and other avian species. APEC are found in the intestinal microflora of healthy birds and most of the diseases associated with them are secondary to environmental and host predisposing factors (Dho-Moulin and Fairbrother, 1999).

The study consisted in the serotyping of n.105 *E. coli* strains, isolated during the period 2000-2004 in Lombardia and Emilia Romagna (North Italy) from poultry and turkeys, reared both in rural and intensive units. These animals represented an heterogeneous picture of the avian population breded in Lombardia and Emilia-Romagna.

*E. coli* strains were isolated from cloacal swabs at one of the n.17 Provincial Diagnostic Sections of the Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia-Romagna, Brescia (IZSLER), using standard bacteriological methods. Further examination for the characterization of the isolated strains i.e. determination of serogroup,
was performed at IZSLER “Specialized Bacteriology Department”.

**Material and methods**

The adopted serotyping technique was the one used in Lugo’s E. coli Reference Laboratory – Spain (Blanco and Blanco, 1993), opportunely adapted according to our laboratory procedures. All the strains were in advance confirmed as E. coli by the Enterotube II Roche Test; then, cultivated on Trypticase Soy Agar (TSA), Mac Conkey Agar and Trypticase Soy Broth (TSB) by successive steps. Each broth culture, developed by only one strain of E. coli, was heated in autoclave for 1 hour at 100 °C and doubled with 0.5% phenolic physiological solution (PPS). Serotyping was carried out using a battery of monospecific antisera towards n.37 different somatic O antigens (O1, O2, O4, O6, O8, O9, O10, O11, O15, O18, O20, O21, O22, O26, O45, O49, O64, O73, O75, O78, O83, O86, O88, O101, O103, O109, O111, O115, O128, O132, O138, O139, O141, O147, O149, O153, O157). These antisera were selected according to national and international literature (Blanco and Blanco, 1993; Farina et al., 1996) and to the present diffusion of O serotypes among domestic animal species in Italy (Farina et al., 1996).

A part of each broth culture (50 µl) was put in contact with the same volume of each antiserum in U bottom polystyrene microtitre plates and cultured for 24 hours at 37 °C in a moist box to cause a slow sero-agglutination (SAL). The broth culture (50 µl) was also put in contact with the same volume of PPS to estimate the auto-aggluti-
nating power of each strain. Negative reactions were indicated by a sharp point, whereas positive reactions by a carpet. The *E. coli* strain was considered non typeable when agglutination was observed in four or more microwells. In addition, titration was carried out if agglutination was observed towards 2 or 3 antisera. The titre was determined by SAL using 50 $\mu$l of the broth culture and 6 base dilutions of the positive antisera and corresponded to the highest positive dilution.

**Results and discussion**

Figures 1 and 2 schematically report the results of O antigens' detection carried out in poultry and turkeys during last five years (2000-2004).

Poultry: O78 (49%), O88 (15%) and O2 (9%) were the serogroups most frequently detected, with a large prevalence over the other serogroups (O157, O149, O141, O111, O103, O101, O64, O22, O8), which made up 27% of the isolates.

Turkeys: of the n. 40 *E. coli* isolates examined, 30% belonged to serogroup O78, 14% to serogroup O8; O141, O103, O88, O21, O20, O11, O9 and O2 were the other identified serogroups, which represented the 56% of the all isolates.

A large number of isolates was untypeable, 49.2% and 65% in poultry and turkeys respectively. The comprehensive results of serotyping for the considered avian species are reported in table 1.

**Conclusions**

In previous reports other Authors indicated a predominance of O78, O88, O8 and O2 serogroups (Gross, 1994; Blanco et al., 1998); in our study O78 was the most frequently recovered serogroup; this datum is in accordance with previous demonstrations (Cloud et al., 1985; Blanco et al., 1998). Concerning the other *E. coli* serogroups identified in this study, they have already been reported from cases of avian colibacillosis (Barnes and Gross, 1997).

We have to consider that many pathogenic isolates do not belong to these identified serogroups, and they are commonly designated as "untypeable"; this results in difficulties in identifying APEC strains in veterinary laboratories.

However, at the moment the characterization of isolated strains, constitutes an indicative suggestion of *E. coli* serogroups mostly diffused in Lombardia and Emilia Romagna (North Italy) regions.

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