

Detection of Shiga toxin-producing *Escherichia coli* O157 in living layer hens

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The present report was undertaken with the aim to evaluate the presence of Shiga toxin-producing *Escherichia coli* (STEC) of the O157 serotype in living layer hens to analyse the role of this avian species as potential reservoir.

This study was carried out between January 2004 and September 2004 on three intensive management layer hen farms (named A, B, C) in the Campania Region (Southern Italy). 60 cloacal swabs were collected from each farm. A total of 180 cloacal swabs were obtained. All cloacal swab samples were enriched 1:10 in 10 ml of modified Tryptone Soy Broth supplemented with novobiocin and incubated at 41 ± 1 °C, for 12-18 h. Subsequently, immunomagnetic separation (IMS) was done. After IMS, the solution was inoculated onto sorbitol MacConkey agar supplemented with cefixime-tellurite and Chromogenic *E. coli* O157 Agar. Suspected *E. coli* O157 colonies were tested by agglutination with an *E. coli* O157 latex test kit for confirmation. *E. coli* O157 isolates were screened for possession of genes encoding for the *stx1* and *stx2*, the *E. coli* attaching-and-effacing (*eae*) and *hly* genes. A polymerase chain-reaction (PCR) with the use of established primers was used.

STEC was detected in 13 (three from farm A and ten from farm C) of the total 180 samples.

All isolates from farm A and farm C carried *eae* and *stx2* genes. Two isolates from farm A and eight isolates from farm C carried *stx1* gene. Additionally, all isolates from farm A and six isolated from farm C carried *hly* gene. In contrast, all isolated from Farm B were negative for STEC.

To our knowledge the present report is the first isolating STEC in living layer hens in Italy. All layer hens analysed in our report were apparently in a healthy body condition and which emphasised our hypothesis about their potential role as reservoir hosts for STEC O157.

Keywords: *Escherichia coli* O157; layer hens; reservoir; Shiga toxin

Introduction

The Shiga toxin-producing *Escherichia coli* (STEC) of the O157 serotype is the cause of a serious human alimentary infection associated with haemorrhagic or watery diarrhoea which may, particularly in children, be complicated by life threatening haemolytic uremic syndrome (Naylor et al. 2005).

Several epidemiological studies have revealed that O157 VTEC strains are prevalent in the gastrointestinal tract of healthy cattle, and therefore cattle are regarded as the principal reservoir of these pathogens (Armstrong et al. 1996).

At present, there is limited information on the prevalence of O157 STEC in live chickens even though this pathogen has been shown to colonise the caecum of chicks for long periods and eggs shell following experimental infection (Schoeni and Doyle 1994).

The aim of this report was to carry out a health status monitoring in order to evaluate the presence of STEC in living layer hens.

Materials and methods

This study was carried out between January 2004 and September 2004 on three intensive management layer hen farms (named A, B, C) in Southern Italy. The population size of farms A, B, and C was 32500, 9000 and 15000 respectively.

60 cloacal swabs were collected from each farm (Thrusfield 1995). A total of 180 cloacal swabs were collected. All cloacal swab samples were enriched 1:10 in 10 ml of modified Tryptone Soy Broth (Oxoid Ltd, Basingstoke, Hampshire, UK) supplemented with novobiocin (Oxoid Ltd) and incubated at 41 ± 1 °C, for 12-18 h. One ml of each culture medium was added to 20 µl of magnetic beads coated with antibody to O157 (Dynal Biotech ASA, Oslo, Norway) and immunomagnetic separation was performed according to the manufacturer's instructions. Finally, the magnetic beads were inoculated onto both sorbitol MacConkey agar (Oxoid Ltd) supplemented with cefixime-tellurite (Oxoid Ltd) and Chromogenic *E. coli* O157 Agar (Biolife italiana S.r.l., Milano, Italy). After incubation at 37° C from 18 to 24 h, sorbitol-negative colonies were selected and screened for the presence of the O157 antigen by agglutination with an *E. coli* O157 latex test kit (Oxoid Ltd).

Escherichia coli O157 isolated were sub-cultured on washed sheep blood plates and incubated overnight at 37° C. During incubation, enterohaemolysin production was evaluated according to Beutin et al. (1996).

All isolates confirmed to be *Escherichia coli* O157 by Chromogenic *E. coli* O157 Agar and latex test kit were subjected to a multiplex polymerase chain reaction (PCR) assay to determine the presence of *stx* (*stx1* and/or *stx2*), the *E. coli* attaching-and-effacing (*eae*) and *hly* genes. The last gene were evaluated to confirm the enterohaemolysin production on washed sheep blood plates.

DNA extraction and Multiplex PCR assay for *stx1*, *stx2*, *eaeA*, *hlyA* genes amplification were performed as previously described by Fagan et al. (1999). Oligonucleotide sequence of primers and the predicted sizes of PCR amplified products are listed in Table 1. A GeneRuler 100bp Ladder Plus (Fermentas International Inc., Burlington, Ontario, Canada) was used.

Table 1 Primers used in multiplex PCR

Primer	Oligonucleotide sequences (5'-3')	Expected size
<i>stx1</i> -F	ACACTGGATGATCTCAGTGG	614bp
<i>stx1</i> -R	CTGAATCCCCCTCCATTATG	
<i>stx2</i> -F	CCATGACAACGGACAGCAGTT	779bp
<i>stx2</i> -R	CCTGTCAACTGAGCAGCACTTTG	
<i>eaeA</i> -F	GTGGCGAATACTGGCGAGACT	890bp
<i>eaeA</i> -R	CCCCATTCTTTTTCACCGTCG	
<i>hlyA</i> -F	ACGATGTGGTTTATTCTGGA	165bp
<i>hlyA</i> -R	CTTCACGTGACCATACATAT	

Results and discussion

As shown by table 2, STEC was detected in 13 (three from farm A and ten from farm C) of the total 180 samples. In particular, all isolates from farm A and farm C carried *eae* and *stx2* genes. Moreover two isolates from farm A and eight isolates from farm C carried *stx1* gene. Additionally, all isolates from farm A and six isolated from farm C carried *hly* gene. In contrast, all isolated from Farm B were negative for STEC.

Table 2 Number and percentage of STEC positive cloacal swab samples collected from three intensive management layer hen farms

Farm	No. of positive samples	% of positive sample
A	3/60	5%
B	0/60	0%
C	10/60	16%
Total	13/180	7,2%

Within layer hen farms A and C we found STEC O157 in all occasions investigated. In contrast, farm B was always found negative. In literature, information about prevalences of STEC within these types of farms is rare. In the Netherlands, Heuvelink et al. (1999) isolated *E. coli* O157 strains from turkey flocks but not from chicken flocks. STEC have not been found in live chickens. Furthermore, during an investigation to study the role of birds as source of STEC carried out by Kobayashi et al. (2002) no shiga toxin samples were detected. However, in poultry, persistent colonization of *E. coli* O157:H7 can occur, following experimental infection, leading to excretion of the pathogen in the faeces (Schoeni and Doyle 1994).

STEC O157 isolated from living layer hens, in this report, can be regarded as important zoonotic pathogens because they possess *hly* gene, which is hallmark of human pathogenic strains (Kobayashi et al. 2002).

All layer hens analysed in our report were apparently in a healthy body condition. Our results could indicate this avian species as possible new reservoir for STEC O157. Therefore, further studies should be conducted to confirm our hypothesis.

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