Survey of Shiga toxin-producing 
*Escherichia coli O157:H7*
 in urban pigeons 
(*Columba livia*)
in the city of Napoli, Italy

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**ABSTRACT**

Recently, several studies have demonstrated that pigeon is an important reservoir of Shiga toxin-producing *E. coli* O157:H7. The aim of this study was to evaluate the presence of this pathogen in urban pigeons in the city of Napoli. The sampling was carried out during the period November 2005/July 2006. The city was subdivided in 56 quadrants by Geographical Information System. Each quadrant was analysed three times. From each quadrant, 3 pigeons were analysed by cloacal swabs. A total of 504 cloacal swabs was obtained. We isolated four *E. coli* O157:H7 strains. By multiplex PCR, all strains carried *eae* and *stx2* genes, whereas only one strain carried the *stx1* gene. 2/4 isolated strains carried *hly* gene which is considered a hallmark of human pathogenic strains. Our results indicate that pigeon faces are a source of *E. coli* O157:H7 for birds, mammals and humans.

**Key words:** Pigeon, *Escherichia coli* O157:H7, Shiga toxins, Zoonotic risk, Napoli.

**RIASSUNTO**

**RICERCA DI E. COLI O157:H7 PRODUTTORE DI SHIGATOSSINE IN PICCIONI URBANI NELLA CITTÀ DI NAPOLI, ITALIA.**

Recenti studi hanno dimostrato che il piccione può fungere da serbatoio di *E. coli* O157:H7 produttore di Shiga tossine. Lo scopo di questo lavoro è stato di valutare la presenza di *E. coli* O157:H7 nei piccioni urbani, considerando l'eventuale rischio zoonosico correlato. Il campionamento è stato eseguito da novembre 2005 a luglio 2006 in tre interventi nella Città di Napoli suddivisa in 56 transetti mediante l'ausilio del Geographical Information System. In ciascun transetto sono stati catturati 3 piccioni ai quali è stato effettuato un tampone cloacale, utilizzato poi in laboratorio per l'isolamento batterico di *E. coli* O157:H7. In totale sono stati eseguiti 504 tamponi cloacali. Tramite procedura operativa standard, sono stati isolati 4 ceppi ascrivibili ad *E. coli* O157:H7. Successivamente, mediante multiplex PCR, i 4 ceppi ottenuti presentavano tutti il gene eae codificante il meccanismo di adesione "attaching and effacing". In aggiunta, tutti i ceppi isolati mostravano il gene codificante la Stx1, mentre solo 1 ceppo presentava il gene codificante la Stx2. Infine, 2 dei 4 ceppi isolati presentavano il gene *hly* codificante l'enteroemolisina e considerato da diversi autori un importante marker di patogenicità per l'uomo. Pertanto, i piccioni possono essere considerati una fonte di *E. coli* O157:H7 per uccelli, mammiferi ed uomo.

**Parole chiave:** Piccione, *Escherichia coli* O157:H7, Shiga tossine, Rischio zoonosico, Napoli.
Introduction

Pigeons (Columbia livia) are widely distributed in urban areas of city of Naples, and come into close contact with humans in parks, temples, shrines, public gardens and railroad stations. A recent increase in the number of pigeons has raised public health concerns (Haag-Wackernagel and Moch, 2004). Pigeons are potential reservoirs for several pathogenic bacteria, including *E. coli* O157:H7. 

Shiga toxin-producing strains of *Escherichia coli* (STEC) O157:H7 occur in the faecal floras of healthy domestic animals and are most frequently found in ruminants (Cizek et al., 1999). STEC infection in humans can cause severe diseases such as hemorrhagic colitis and haemolytic-uramic syndrome (Wallace et al., 1997). Cattle are now considered to be the principal source of *Escherichia coli* O157:H7 causing human disease; transmission may occur through a variety of routes (Naylor et al., 2005). In addition, STEC have also been isolated from other mammals and birds. This pathogen has been shown to colonise the intestinal tract of pigeons for long period following experimental infection (Cizek et al., 2000). Morabito et al. (2001) have described the presence of STEC strains in the stools of feral pigeons in the city of Rome. Those strains possessed the *eae* gene and produced a variant of Stx2 which was characterised and designated Stx2f (Schmidt et al., 2000). Therefore, the present study was undertaken with the aim to evaluate the presence of Shiga toxin-producing *Escherichia coli* O157:H7 in urban pigeons in the city of Naples.

Material and methods

This study was carried out in the city of Naples from November 2005 to July 2006. The city was subdivided in 56 quadrants by Geographical Information System (GIS) as described by Maguire, 1991. The sides of each quadrant measured 1400x2000m. In each quadrant 3 pigeons were sampled to isolate STEC by cloacal swabs. During the sampling each quadrant was analysed three times (November 2005, March 2006, July 2006). All cloacal swab samples were enriched 1:9 in 9ml of modified Tryptone Soy Broth (Oxoid Ltd, Basingstoke, Hampshire, UK) supplemented with novobiocin (Oxoid Ltd) and incubated at 41±1°C, for 12-18h. 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 24h, sorbitol-negative colonies were selected and screened for the presence of the O157 and H7 antigens by agglutination with an *E. coli* O157:H7 Latex Test (Remel, Lenexa, KS USA). *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 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 Kim et al. (2005). Oligonucleotide sequence of primers (Fagan et al., 1999) 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.
sent a source of \textit{E. coli} O157:H7.

We believe that continuous surveys can estimate, and thus help to minimise the risk of human contracting diseases from pigeons.

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Beutin, L., Knollmann-Schanbacher, G., Rietschel, W., and Seeger, H., 1996. Animal reservoirs of \textit{Escherichia coli} O157: H7 Latex Test (Remel). As shown by PCR, all STEC isolated carried \textit{eae} and \textit{stx2} genes. Moreover one isolate carried \textit{stx1} gene. Additionally, two isolates carried \textit{hly} gene. STEC was isolated in a high density urban quadrant corresponding the Chiaia-Posillipo-San Ferdinando area. STEC have been reported in pigeons from several parts of the world. In Italy, Morabito \textit{et al.} (2001) isolated STEC from 70/649 pigeons sampled but \textit{E. coli} O157 serotype was not found from any of the samples. In Japan, in a study conducted by Tanaka \textit{et al.} (2005) all 108 faecal samples examined were negative for \textit{E. coli} O157. Finally, in Norway, Cizek \textit{et al.} (1999) isolated 0/50 STEC.

\textbf{Conclusions}

STEC O157:H7 isolated from pigeons, in this report, can be regarded as important zoonotic pathogens because they posses \textit{hly} and \textit{Stx2} genes, which are hallmark of human pathogenic strains (Kobayashi \textit{et al.}, 2002). Therefore, the present results suggest that pigeon faeces represent a source of \textit{E. coli} O157:H7.

We believe that continuous surveys can estimate, and thus help to minimise the risk of human contracting diseases from pigeons.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{Primer} & \textbf{Oligonucleotide sequences (5’-3’)} & \textbf{Expected size} \\
\hline
\textit{stx1}-F & ACACTGGATGATCTCAGTGG & 614bp \\
\textit{stx1}-R & CTGAATCCCCCTCCATTAG & 779bp \\
\textit{stx2}-F & CCATGACAACGGACAGCAGTT & 779bp \\
\textit{stx2}-R & CCGTGAACGTGAGCAGCTTGG & 890bp \\
\textit{eaeA}-F & GTGCGGAAATCTCGCGAGACT & 890bp \\
\textit{eaeA}-R & CCCCATTTTTTCCCCACGC & 165bp \\
\textit{hlyA}-F & ACAGATGGTGTTATTTCTGGA & 165bp \\
\textit{hlyA}-R & CCTCACGTGACCACATCATAT & 165bp \\
\hline
\end{tabular}
\caption{Primers used in multiplex PCR.}
\end{table}

\textbf{Results and discussion}

The distribution of STEC-positive cloacal swab samples of the quadrants considered was four out of the 504 samples. All strains isolated were positive to O157 and H7 antigens by agglutination with an \textit{E. coli} O157:H7 Latex Test (Remel). As shown by PCR, all STEC isolated carried \textit{eae} and \textit{stx2} genes. Moreover one isolate carried \textit{stx1} gene. Additionally, two isolates carried \textit{hly} gene. STEC was isolated in a high density urban quadrant corresponding the Chiaia-Posillipo-San Ferdinando area. STEC have been reported in pigeons from several parts of the world. In Italy, Morabito \textit{et al.} (2001) isolated STEC from 70/649 pigeons sampled but \textit{E. coli} O157 serotype was not found from any of the samples. In Japan, in a study conducted by Tanaka \textit{et al.} (2005) all 108 faecal samples examined were negative for \textit{E. coli} O157. Finally, in Norway, Cizek \textit{et al.} (1999) isolated 0/50 STEC.

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