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ScienceDirect
International Journal of Food Microbiology 113 (2007) 237–241
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Short Communication

# Isolation and characterization of Shiga toxin-producing *Escherichia coli* O157:H7 and non-O157 from beef carcasses at a slaughter plant in Mexico

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Received 28 April 2005; received in revised form 14 March 2006; accepted 13 June 2006

**Abstract**

The contamination of beef carcasses with Shiga toxin-producing O157:H7 and non-O157 *Escherichia coli* (STEC) obtained from a slaughter plant in Guadalajara, Mexico was investigated. A total of 258 beef carcasses were sampled during a 12-month period. All samples were assayed for STEC by selective enrichment in modified tryptone soy broth supplemented with cefixime, cefsulodin and vancomycin, followed by plating on Sorbitol MacConkey Agar supplemented with cefixime and tellurite (CT–SMAC). Simultaneously, all samples were assayed by immunomagnetic separation (IMS) and plated on CT–SMAC and CHROMagar®. The presence of the *stx1*, *stx2*, *eaeA* and $hly_{933}$ genes, recognized as major virulence factors of STEC, was tested for O157:H7 and non-O157 *E. coli* isolates by multiplex polymerase chain reaction (PCR). STEC was detected in two (0.8%) samples. One of these STEC isolates corresponded to the serotype O157:H7 showing *stx2*, *eaeA* and $hyl_{933}$ genes. The other isolate corresponded to non-O157 STEC and only had the *stx1* gene. Thirteen carcasses (5%) were positive for nonmotile *E. coli* O157 and 7 (2.7%) were positive for *E. coli* O157:H7. The presence of O157:H7 and non-O157 STEC on beef carcasses in this slaughter plant in Guadalajara, Mexico, emphasizes the importance of implementing the Hazard Analysis and Critical Control Point (HACCP) system, as well as the need for implementing, evaluating, and validating antimicrobial interventions to reduce the presence of potential pathogenic microorganisms.


*Keywords:* *Escherichia coli* O157:H7; *Escherichia coli* non-O157; Carcasses; Beef; Mexico

## 1. Introduction

*Escherichia coli* O157:H7 was first identified as a human pathogen capable of causing food-borne disease in 1982 (Riley et al., 1983). Common symptoms caused by *E. coli* O157:H7 include hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (Karmali, 1989). Since 1982, several outbreaks of hemorrhagic colitis and hemolytic uremic syndrome associated with this pathogen have been reported worldwide following the ingestion of contaminated foods of bovine origin (Belongia et al., 1991; O'Brien et al., 1993; Chapman et al., 1993). Cattle are an important reservoir of *E. coli* O157 and non-O157 STEC (Wells et al., 1991; Whipp et al., 1994; Bettelheim, 2000). The microorganism is carried as part of the native microbiota in the intestine of cattle and can contaminate meat and the slaughterhouse environment (Bell, 1997). Feces and hides are significant sources of bacterial carcass contamination (Elder et al., 2000; McEvoy et al., 2000; Gun et al., 2003), however antimicrobial interventions can significantly reduce the number of bacteria of fecal origin on beef carcasses (Castillo et al., 1998, 1999). The aim of this study was to determine the occurrence of Shiga toxin-producing *E. coli* O157:H7 and non-O157 on beef carcasses obtained from a

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0168-1605/$ - see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2006.06.028

slaughterhouse in Guadalajara city, Mexico and to characterize these isolates based on the presence of *stx1*, *stx2*, *eaeA* and $hly_{933}$ genes.

## 2. Materials and methods

### 2.1. Characteristics of the slaughterhouse and cattle

All samples were obtained from a municipal slaughter plant located in the city of Guadalajara, Mexico. This facility does not meet the requirements for being federally inspected. The slaughter plant processes approximately 500 heads per day at a chain speed of 65 animals per hour. After carcass evisceration and splitting, two water rinses are applied to remove gross contamination. During the slaughter process no antimicrobial interventions are applied.

### 2.2. Sample collection

Surface samples from the sides of refrigerated carcasses ($n=258$) were randomly collected at least 12 h after slaughter on a weekly basis during a 12-month period. All samples were collected using sterile polyurethane sponges (BioPro® Sampling System) moistened with 25 ml of buffered water. Each sample was taken by scrubbing a 100-$cm^2$ area at three different sites which included rump, flank, and brisket regions of each carcass side, for a total area of 300-$cm^2$ (FSIS, 1996). Sponges were placed in sterile bags (Whirl-Pak®) and packed in pre-chilled insulated containers for transportation to the laboratory for testing between 4 and 24 h after sampling.

### 2.3. Isolation and identification of E. coli O157 and non-O157

All samples were examined for the presence of *E. coli* O157 and non-O157 using a selective enrichment procedure followed by a direct plating procedure on CT–SMAC. Simultaneously all samples were examined by immunomagnetic separation (IMS) followed by plating on CT–SMAC and CHROMagar®. Each sample was placed in a stomacher bag and enriched with 225 ml of modified tryptone soy broth (Becton Dickinson and Co. Sparks, MD, USA) supplemented with cefixime (0.05 mg/l, Dynal Biotech, Norway), cefsulodin (10.0 mg/l, Sigma-Aldrich, USA) and vancomycin (8.0 mg/l, Sigma-Aldrich, USA), pummeled for 2 min with a stomacher blender (BagMixer® 400 ml Interscience, France) and then incubated at 37 °C for 24 h.

#### 2.3.1. Enrichment-plating procedure

After 6 and 24 h of incubation, each enriched sample was pummeled as previously described. Appropriate decimal dilutions of the enrichment were performed in 0.1% peptone water and 0.1-ml aliquots were plated on Sorbitol MacConkey Agar (Becton Dickinson and Co. Sparks, MD, USA) supplemented with cefixime and potassium tellurite (50 ng/ml and 25 mg/ml respectively; Dynal Biotech supplement) (CT–SMAC). Additionally, one loopful of the enrichment broth was directly streaked on CT–SMAC. All plates were incubated at 37 °C for 18–24 h (FDA, 1998).

#### 2.3.2. Immunomagnetic separation (IMS) procedure

After 6 h of incubation, 1-ml aliquots from each enrichment were used to perform IMS with Dynabeads® anti-*E. coli* O157 (Dynal Biotech, Oslo, Norway, code 710.04), according to the procedure recommended by the manufacturer. 50-µl aliquots of the IMS-enriched material were streaked on CT–SMAC and CHROMagar O157 (Dynal Biotech, Norway). All plates were incubated at 37 °C for 18–24 h.

#### 2.3.3. Biochemical and serological tests for identification of isolates

Suspected *E. coli* O157:H7 colonies were selected for biochemical identification using the API 20E system (bioMérieux, France). Confirmed *E. coli* isolates were tested for O157 and H7 antigens by agglutination procedures. Those isolates which tested negative for the H7 antigen were examined for motility by wet-mount microscopy. Nonmotile isolates with a positive agglutination test for O157 antigen were considered as *E. coli* O157:NM. *E. coli* O157:H7 ATCC 43895 reference strain was used as positive control for both biochemical and serological tests.

#### 2.3.4. Identification of virulence factors

*E. coli* O157:H7, *E. coli* O157:NM and *E. coli* non-O157 isolates were subjected to multiplex polymerase chain reaction (PCR) assay to determine the presence of Shiga toxin *stx1* and *stx2*, intimin (*eaeA*) and hemolysin ($hly_{933}$) genes, which are recognized as major virulence factors of STEC. The primers used for *stx1* and *stx2* were previously described by Meng et al. (1997). Primers selected for *eaeA* and $hly_{933}$ correspond to those described by Fratamico et al. (1995) and Fratamico and Strobaugh (1998). All primers were obtained form Invitrogen, Life Technologies, Cal. USA. PCR conditions were those described by Fratamico et al. (2000) and the DNA template was prepared as indicated by Feng and Monday (2000). *E. coli* O157:H7 ATCC 43895 reference strain was used as positive control. Products from the multiplex PCR were visualized following electrophoresis through 1.5% agarose gels which were stained with ethidium bromide.

### 2.4. Statistical analysis

Results were reported as percentage of carcasses testing positive for *E. coli* O157 and *E. coli* non-O157. A Student *T*-test was performed to compare the frequency of isolation during cold and warm seasons. All statistical analyses were conducted using SPSS 11.1.

## 3. Results and discussion

*E. coli* non-O157, *E. coli* O157:NM and *E. coli* O157:H7 were isolated from 53 (20.5%), 13 (5%) and 7 (2.7%) respectively of the 258 beef carcasses sampled in this study. Several studies have reported the prevalence of *E. coli* O157:H7 on beef carcasses. Barckocy-Gallagher et al. (2003) recovered *E. coli* O157:H7 from 1.2% carcasses that were sampled at chilling step and after application of an antimicrobial intervention in commercial beef processing plants in the USA, Elder