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The occurrence of *Escherichia coli* O157 in/on faeces, carcasses and fresh meats from cattle

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ABSTRACT

The aim of this study was to investigate whether *Escherichia coli* O157 is present in/on raw beef in Serbia. Correlated faecal and carcasses samples from 115 slaughtered cattle plus 26 uncorrelated carcass samples were examined. *E. coli* O157 detection and identification was performed using selective enrichment and immunomagnetic separation followed by selective media-plating and biochemical tests.

The *E. coli* O157 occurrences were 2.6% in faeces and 2.8% on carcasses. The *E. coli* O157 occurrences were 0%, 6.2% and 2.1%, respectively, in 106 samples of beef trimmings, 48 samples of minced beef and 48 samples of batter intended for production of raw, fermented sausages. The results confirmed that faecal contamination is very important for the occurrence of *E. coli* O157 on beef carcasses. Furthermore, the present study revealed occasional presence of the pathogen in raw materials used for producing raw, fermented beef sausages.

1. Introduction

Escherichia coli O157 is an important foodborne pathogen, a member of the so-called enterohaemorrhagic or verocytotoxigenic *E. coli* (EHEC, VTEC). Since the early 1980s, *E. coli* O157 have caused a number of human infections (outbreaks or sporadic cases) via foods of animal origin, particularly those originating from cattle e.g. minced/comminuted beef (Anonymous, 1994; Anonymous, 1997; Keene et al., 1997; Pennington, 1998; Williams et al., 2000) and dairy products (Bielaszewska et al., 1997; Curnow, 1999; Morgan et al., 1993; O'Brien & Smith, 1999). Cattle are the major reservoir of the pathogen which is carried in and excreted from their gastrointestinal tract without any symptoms of disease (Chapman, Cerdan Malo, Ellin, Ashton, & Harkin, 2001; Hancock et al., 1998; Renter, Sargeant, Oberst, & Samadpour, 2003).

Faeces and hides of cattle are considered to be the main sources of *E. coli* O157 contamination of carcasses during slaughter (Aslam et al., 2003; Barkocy-Gallagher et al., 2003; Elder et al., 2000). The prevalence of *E. coli* O157 in faeces of cattle at evisceration can vary widely e.g. from 0.0% to 27.8% (Bonardi, Maggi, Pizzin, Morabito, & Caprioli, 2001; Elder et al., 2000; Heuvelink et al., 1998; Minihan, O'Mahony, Whyte, & Collins, 2003; Ransom et al., 2002; Tutenel et al., 2002). *E. coli* O157 occurrences on hides of cattle at slaughter in different countries also vary widely e.g. from 4.5% (Barham et al., 2002) to 56% (Tutenel, Pierard, Van Hoof, & De Zutter, 2003) with occurrence in Serbia reported as 28.2% (Nastasijevic, Mitrovic, & Buncic, 2008). With respect to hide, it is important to note that extensive between animals cross-contamination with *E. coli* O157 takes place before dressing of slaughtered cattle i.e. during the lairage-to-stunning phase, through animal–animal and/or animal–environment–animal contact (Collis et al., 2004).

Contamination of carcass meat with *E. coli* O157 can occur during dressing; primarily during the skinning but also during the evisceration phase. Reported *E. coli* O157 occurrences on beef carcasses vary widely, from 1.1% to 43.4% (Elder et al., 2000; Lahti, Ruoho, Rantala, Hanninen, & Honkanen-Buzalski, 2003; McEvoy et al., 2003; Minihan et al., 2003; Tutenel et al., 2003). In beef samples at retail level, reported *E. coli* O157 occurrences usually range from 0% to 9% (Brooks et al., 2001; Doyle & Schoeni, 1987; Suthienkul et al., 1990; Tutenel et al., 2003; Vuddhakul et al., 2000) although an exceptionally high occurrence of 36% was also published (Radu et al., 1998). Published data on *E. coli* O157 prevalence in raw materials intended for producing raw, fermented beef sausages are scarce; an occurrence of 0.12% in minced beef at meat processing level was reported (Vernozy-Rozand et al., 2002).

Presently, there are no published data on whether, and with which frequency, *E. coli* O157 is present on/in raw beef in Serbia. Therefore, the main aim of the present study was to obtain the first

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scientific information on contamination of beef carcasses and minced/comminuted beef to be used towards baseline data and optimisation of control strategies for *E. coli* O157 in the beef chain.

2. Materials and methods

2.1. General sampling plan

During 12 visits (in winter and spring), correlated samples of faeces and dressed carcasses from each of 115 slaughtered cattle, plus carcass-only samples from 26 animals, were collected in a commercial abattoir (abattoir I; processing up to 100 cattle per day). Furthermore, during two visits, samples of beef trimmings (usually used for minced meat) were collected from 106 beef carcasses in two other abattoirs (70 in abattoir II, 36 in abattoir III). Each trimmings sample comprised of pooled small pieces of surface meat (each approximately 25 cm^2) removed (trimmed) from four regions (rump, flank, brisket and neck) of a half-carcass. Also, in abattoir III, 48 samples of minced beef (the meat originating from 15 animals) and 48 samples of complete sausage batter (minced beef with added nitrite-salt mixture, gluconodeltalactone and spices; the meat originating from 23 animals), both intended for production of raw, fermented sausages, were collected.

2.2. Sampling of faeces

The terminal rectum (at the recto-anal junction) containing faecal material was cut from the gastrointestinal tract of each sampled slaughtered bovine and individually placed in a stomacher bag (19 × 30 cm, Nasco, Whirl-pak, USA) and transported on ice (in cool-bin) to the laboratory within 2–3 h.

2.3. Sampling of carcasses

In the abattoirs involved in this study, similarly to usual practices in both Serbia and the EU, no carcass decontamination treatments were used. Each carcass, after dressing but before final wash and chilling, was inspected for presence/absence of any visible faecal contamination by visual examination, and then sampled. Sponge-swab samples were collected from alternate left and right half-carcasses. Plain cellulose washing-up sponges (10 × 10 cm; 2 cm thick) not containing antimicrobial agents (Reid, Avery, Hutchinson, & Buncic, 2002) were exposed to UV light for 15 min and aseptically wrapped in sterile aluminium foil until use. Before use, each sponge was wetted with 10 ml of sterile maximum recovery dilluent (MRD, Oxoid, Hampshire, England, UK) and the rump-hock-flank-brisket area (approximately 2000 cm^2) of each half-carcass was swabbed with a single sponge. Subsequently, each sponge was placed in a stomacher bag and transported on ice (in cool-bin) to the laboratory within 2–3 h.

2.4. Sampling of beef trimmings, minced beef and sausage batter

Each sample (approximately 50 g) was placed in a stomacher bag and transported on ice (in cool-bin) to the laboratory within 2–3 h.

2.5. Homogenization of samples

In the laboratory, the 90 ml of MRD was added to each stomacher bag with sponge- samples taken from carcasses (1:1), whilst the samples of faeces, beef trimmings, minced beef and sausage batter were individually weighed and a 9-fold amount of MRD was added to each stomacher bag. The stomacher bags were manually massaged for 1 min to obtain the homogenates; these were further decimally diluted in MRD.

2.6. Isolation of E. coli O157

The procedure essentially followed the ISO method for *E. coli* O157 (ISO 16654:2001).

Firstly, 25 ml was taken from each homogenate and added to 225 ml modified tryptone soya broth base (mTSBn; Oxoid, Hampshire, England, UK) containing bile salts, K_2HPO_4 (1.5 g/l; Sigma) and novobiocin (5 mg/ml; Sigma). The broth was incubated for 24 h at 41.5 °C, for enrichment.

Secondly, *E. coli* O157 cells were concentrated and separated from the enrichment broth by the immunomagnetic separation technique (IMS; Chapman, Wright, & Siddons, 1994; Wright, Chapman, & Siddons, 1994) using the Dynabeads kit according to the manufacturer's instructions (Dynal, Oslo, Norway).

Thirdly, the separated microorganisms were plated onto sorbitol MacConkey agar containing cefixime (25 μl/500 ml: Dynal, Oslo, Norway) and potassium tellurite (25 μl/500 ml: Dynal) [CT-SMAC], as well as onto sorbitol MacConkey agar containing cefixime (25 μl/500 ml: Dynal) and rhamnose (2.5 g/500 ml; Sigma) [CR-SMAC]. The plates were incubated for 24 h at 37 °C, and up to three suspect colonies were selected per plate (colourless or with dark pink centers) for confirmation, sub-cultured onto plate count agar (PCA; Oxoid) and incubated for 24 h at 37 °C.

Fourthly, suspect colonies were confirmed as *E. coli* using a biochemical identification kit (API 20E, BioMerieux, France) and as serotype O157 by using DrySpot O157 latex agglutination test- (Oxoid, Hampshire, England, UK).

2.7. Determination of generic E. coli

The procedure essentially followed the ISO method for *E. coli* (ISO 16649:2001). Briefly, 1 ml amounts of appropriate dilutions of each sample homogenate were plated (double-plates) on cellulose membrane overlaid on Mineral-modified-glutamate agar (MMGA; Merck, Germany). The plates were incubated at 37 °C for 4 h and the membranes were transferred onto plates of Tryptone-bile-glucuronide agar (TBX; Merck, Germany). The plates were incubated at 44 °C for 18–24 h and typical (blue) colonies were counted.

2.8. Determination of Enterobacteriaceae

The procedure essentially followed the ISO method for *Enterobacteriaceae* (ISO, 21528-2:2004). Briefly, 1 ml amounts of appropriate dilutions of each sample homogenate were plated (double-plates) on Violet Red Bile Glucose Agar (VRBG agar; Merck, Germany). The plates were incubated at 37 °C for 24 h, colonies counted (detection limit 5.0×10^{-2} CFU/g) and up to five presumptive colonies per plate were sub-cultured and confirmed as *Enterobacteriaceae* using API 20E kit (Biomerieux, France).

2.9. Determination of Total Viable Counts

The procedure essentially followed the ISO method for Total Viable Counts (ISO, 4833:2003). Briefly, 1 ml amounts of appropriate dilutions of each sample homogenate were plated (double-plates) on Plate Count Agar (PCA; Merck, Germany). The plates were incubated aerobically at 30 °C for 72 h and the colonies were counted (detection limit 5.0×10^{-2} CFU/g).

2.10. Results analysis

Where applicable, bacterial counts per g or cm^2 were converted into logarithms before calculating mean values, standard deviation and significance of differences between means, using a statistical package (SPSS).