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Transfer of verocytotoxigenic *Escherichia coli* O157, O26, O111, O103 and O145 from fleece to carcass during sheep slaughter in an Irish export abattoir

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ABSTRACT

The purpose of this study was to investigate carriage and transfer of verocytotoxigenic *Escherichia coli* (VTEC) O157, O26, O111, O103 and O145 from fleece to dressed carcasses of 500 sheep, and to establish the virulence potential of recovered VTEC. Individual sheep were tracked and sampled (10 g fleece, full carcass swab) through the slaughter process. Samples were examined for the presence of verotoxin (*vt1* and *vt2*) genes using a duplex real-time PCR assay and positive samples were further screened for the presence of the above five serogroups by real-time PCR. VTEC cells were recovered from PCR positive samples by serogroup specific immunomagnetic separation and confirmed by serogroup specific latex agglutination and PCR. Isolates were subject to a virulence screen (*vt1*, *vt2*, *eaeA* and *hlyA*) by PCR and isolates carrying *vt* genes were examined by Pulsed-Field Gel Electrophoresis (PFGE). VTEC O26 was recovered from 5/500 (1.0%) fleece and 2/500 (0.4%) carcass samples. VTEC O157 was isolated from 4/500 (0.8%) fleece samples and 3/500 (0.6%) carcass samples. *E. coli* O103 was recovered from 84/500 (16.8%) fleece and 68/500 (13.6%) carcasses, but only one *E. coli* O103 isolate (0.2%) carried *vt* genes. *E. coli* O145 was recovered from one fleece sample, but did not carry *vt* genes. *E. coli* O111 was not detected in any samples. For the four serogroups recovered, the direct transfer from fleece to carcass was not observed with PFGE showing that VTEC O26 isolates from a matched fleece/carcass "pair" were not identical. This study shows that while VTEC O157 are being carried by sheep presented for slaughter in Ireland, other potentially clinically significant verotoxin producing strains (particularly VTEC O26) are emerging.

1. Introduction

Escherichia coli O157:H7 and other verocytotoxigenic *E. coli* (VTEC) have the potential to cause illness in humans, ranging from mild non-bloody diarrhoea to more severe and life threatening conditions like haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) (Karmali, 1989; Hussein, 2007). Pathogenicity of VTEC is associated with several genetic markers which include those coded by the verocytotoxin genes *vt1* and *vt2*, *eaeA* (which codes the protein Intimin, aiding the formation of attaching and effacing lesions) and *hlyA* (enterohaemolysin) (Nataro and Kaper, 1998; Paton and Paton, 1998a). In Ireland, the total numbers of reported VTEC infections have previously increased each year with a crude incidence rates rising from 3.9/100,000 in 2007, to 5.3/100,000 in 2008, and of 5.7 per 100,000 in 2009 with a decrease in 2010 to 4.7/100,000 (HSPC, 2011). Although such rates are relatively low in comparison with other food-borne pathogens, the low infectious dose, and the severity of symptoms associated with VTEC infection, make these organisms a significant public health concern (HSPC, 2011).

E. coli O157:H7 is still the best known VTEC serotype, but other serogroups such as *E. coli* O26, O111, O103 and O145 are being increasingly recognised as highly significant threats to public health (Karmali et al., 2003; Johnson et al., 2006). Thus, non-O157 serogroups are being more increasingly frequently reported, and accounted for 41% of all VTEC infections in Ireland in 2010 (HSPC, 2011).

Although cattle are traditionally considered the major reservoir of VTEC (Montenegro et al., 1990; Beutin et al., 1993; Gyles, 2007), other ruminant species, especially sheep, have been increasingly suspected as reservoirs of VTEC infection (Licence et al., 2001; Strachan et al., 2001). A UK study noted *E. coli* O157 was more common in lamb products than beef or mixed meat products (Chapman et al., 2000).

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As is the case with cattle, sheep slaughter is seen as a high risk stage for transfer of these pathogenic micro-organisms from fleece to carcasses and subsequently derived food products (Sheridan, 1998). A number of studies have investigated the prevalence of *E. coli* O157 in sheep and lamb faeces on the farm (Rey et al., 2003; Ogden et al., 2005) at slaughter (Urdahl et al., 2002; Battisti et al., 2006) and on fleece and carcass surfaces (Lenahan et al., 2007) but very little information is available in relation to the presence/significance of non-O157 VTEC in sheep and sheep products. This may be due to the relatively limited availability of effective methods for the recovery and detection of non-O157 VTEC. However, the recent emergence of commercially available diagnostic kits for non-O157 VTEC (including serogroup specific immunomagnetic separation (IMS) beads and latex agglutination kits) has increased the isolation of non-O157 *E. coli*. Similarly, improvements in polymerase chain reaction (PCR) primers and systems, are beginning to facilitate better identification and differentiation of VTEC, more comprehensive recognition of diverse VTEC genotypes associated with human infections (Mackay, 2004) and the relationships between pathogenic VTEC genes (*vt1*, *vt2*, *eaeA* and *hlyA*), and disease severity.

This study therefore aimed to investigate the incidence, numbers and transfer of potentially clinically significant non-O157 VTEC (O26, O111, O103 and O145) along with VTEC O157 in the Irish sheep slaughter chain.

2. Materials and methods

2.1. Bacterial strains

Toxigenic strains of *E. coli* O157:H7, *E. coli* O26, *E. coli* O111, *E. coli* O103 and *E. coli* O145 (Table 1) and *Salmonella* Typhimurium LT2 (NCTC 12416 – Health Protection Agency, London, U.K.) were maintained on Protect™ Stock Culture Beads (Langenbach Services Ltd, Bray, Ireland) at −20 °C. Protect beads coated with each strain were placed into 10 ml of Brain Heart Infusion broth (BHI) (Oxoid, Basingstoke, UK) and incubated for 24 h at 37 °C. The resulting cultures were included as controls in the analysis.

2.2. Abattoir and sampling

Polyurethane sponges swabs (100 mm × 100 mm × 10 mm) (Sydney Heath & Son Ltd, Staffordshire, UK) were prepared in sterile autoclavable bags (Sardstedt, Nümbrecht, Germany). Each sponge was pre-moistened with 10 ml of maximum recovery diluent (MRD, Oxoid), the bags sealed with autoclave tape and autoclaved at 121 °C for 15 min. Forty pairs of stainless steel scissors were placed in individual autoclave bags and sterilised by autoclave (121 °C for 15 min).

Samples were recovered from a commercial sheep export abattoir (chosen to fairly represent similar sized abattoirs throughout Ireland) which slaughtered at a line speed of approximately 260–280 animals per hour, over an 11-month period (June 2008–April 2009). The pelt was pulled from the shoulders manually and removal was completed with a fleece puller. Following dressing and grading, carcasses were washed with potable water at a temperature of 15–40 °C.

During each visit to the abattoir 20–40 animals were tagged and tracked through the slaughter process, by labelling the carcass hook when fleece was taken, for a combined total of 500 tracked sheep over time. Two samples were taken from each animal – a 10 g fleece sample cut from the rump immediately after slaughter and a full external surface carcass swab taken post-evisceration. Environmental samples were also taken from inside the abattoir (swabs of cutting equipment and carcass hooks) as well as faecal samples from the holding pens. All samples were chilled (5–10 °C), transported to the laboratory within 2 h of sampling and stored at 4 °C until processing.

Table 1
Escherichia coli strains, genotypes and sources.

Serogroup	Strain code	Genotype		Origin
		vt1	*vt2*	
O157	380–94	+	+	Food
O26	361	+	–	Clinical
O111	378	+	+	Food
O103	MB2652	+	–	Clinical
O145	MB2692	+	–	Clinical

2.3. Enrichment of samples

The methods used in this study are summarised in Fig. 1. Carcass swab samples were stomached (260 rpm) (BagMixer 400, Interscience, France) for 1 min in 100 ml of pre-warmed (to 37 °C) modified Tryptone Soya Broth (mTSB, Oxoid). The 10 g fleece samples were stomached in 90 ml of pre-warmed mTSB supplemented with vancomycin (6 mg/l) (Sigma Chemical Company, MO, USA) and cefixime (50 μg/l) (Sigma). After stomaching, approximately 3 ml of inoculated mTSB from each sample was aseptically removed and stored at 4 °C for subsequent recovery of VTEC.

The remaining mTSB swab/fleece suspensions were incubated at 37 °C for 6 h, stomached (260 rpm) for 1 min, and 20 ml aliquots were centrifuged at 500 g (Eppendorf 5810R, Eppendorf, Germany) for 10 min to sediment debris. Samples (1 ml), from each supernatant were transferred into a 1.5 ml microcentrifuge tube and centrifuged at 8000 rpm for 10 min. The supernatant was discarded and the resultant cell pellet processed to recover bacterial DNA.

2.4. DNA extraction

DNA was extracted from each bacterial pellet using the DNeasy Blood & Tissue Kit and protocol for the extraction of DNA from Gram-negative bacteria (Qiagen, Crawley, West Sussex, UK) and eluted in 100 μl AE buffer. DNA purity and concentration from each sample was tested using NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The DNA samples were analysed immediately by real-time PCR, or frozen at −20 °C for subsequent analysis.

2.5. Real-time PCR detection

2.5.1. Duplex real-time PCR screen for detection of vt1 and vt2 genes

Duplex real-time PCR reactions were performed on DNA extracts using the LightCycler 2.0 (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) to detect *vt1* and *vt2* genes using *vt* specific TaqMan probes and primers (Table 2). The TaqMan probes were designed and made by Tib-Molbiol (Berlin, Germany). The *vt1* probe was labelled with the fluorescent dye FAM and a blackberry quencher dye and the *vt2* probe was labelled with the fluorescent dye Yakima Yellow and a blackberry quencher dye. The *vt1* and *vt2* primers were made by MWG Biotech AG (Ebersberg, Germany). Duplex amplifications were performed on each DNA extract using the LightCycler® TaqMan® Master kit (Roche Diagnostics GmbH). Each reaction consisted of 4 μl of 5X LightCycler TaqMan MasterMix (Roche Diagnostics GmbH), 1 μl of each TaqMan probe (final concentration 0.1 μM), 1 μl of each *vt1* primer set (final concentration 0.5 μM), 0.5 μl of each *vt2* primer set (final concentration 0.25 μM) and 2 μl of template DNA (≤200 ng/reaction) made