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Survival of *Escherichia coli* O157:H7 and non-pathogenic *E. coli* on irradiated and non-irradiated beef surfaces

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ABSTRACT

This study examined changes in numbers of pathogenic (PEC) and non-pathogenic (NPEC) *Escherichia coli* during storage at 10 °C on the surfaces of irradiated (IR) and non-irradiated (NIR) meat pieces excised from the neck, brisket and rump of beef carcasses and in Brain Heart Infusion Broth (BHI) and Maximum Recovery Diluent (MRD). On irradiated meat pieces, there were significant differences between mean PEC and NPEC counts at all sites. Differences in counts were also observed between IR and NIR surfaces and among the three meat sites for both *E. coli* types. These differences occurred only on IR samples, suggesting that the irradiation associated reductions in normal beef surface flora influenced survival of both *E. coli* types. PEC and NPEC counts increased during storage in BHI, but only NPEC counts increased in MRD. The results of this study highlight the impact of meat surface type and the presence/absence of the normal beef carcass surface flora on *E. coli* survival and/or growth during meat storage. Such previously unreported effects, and their precise mechanisms, have direct implications in the development and application of accurate models for the prediction of the safety and shelf life of stored meat.

1. Introduction

Effective prediction of the bacteriological safety of chilled meats requires accurate information on the numbers/types of pathogenic bacteria present, and their interactions with the contaminated meat surfaces during chill storage. However, valid concerns about the experimental introduction of pathogens into food environments mean that it is rarely possible to directly observe such interactions under commercial conditions. Thus many studies have used indicator organisms (Prendergast, Rowe, & Sheridan, 2007), which can provide useful data, but may give less information of real pathogen food interactions.

A number of broth based studies (Eblen, Annous, & Sapers, 2005; Salter, Ross, & McMeekin, 1998) have suggested that non-pathogenic *E. coli* strains can be used as indicators in modelling the growth of pathogenic *E. coli* O157:H7, and in evaluating the efficacy of interventions designed to reduce the persistence/numbers of this pathogen during meat processing and storage. In more general terms, the use of data from broth experiments to predict *E. coli* growth and survival on beef surfaces may be unreliable, because pathogens in broths face different challenges from those posed by meat surfaces. Food structure may have a profound effect on pathogen growth and this has been demonstrated for *Listeria monocytogenes*, where significant differences in growth were demonstrated between a broth and a gel system and a broth and an agar surface (Wilson et al., 2002; Koutsoumanis, Kendall, & Sofos, 2004). The influence of surface structure in relation to meat spoilage has also been recognised (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

For example, a number of recent studies have demonstrated that survival rates of (non-pathogenic) *E. coli* are different at different sites on beef carcasses (Prendergast and Sheridan, unpublished; Kinsella, 2008). As well as the above physical and environmental factors, pathogen survival and growth has been shown to be significantly influenced by the presence and nature of the natural flora of meat. Thus, Nissen, Maugesten, and Lea (2001) demonstrated more growth of *E. coli* O157:H7 on heat treated, lactic acid decontaminated beef pieces than on untreated control samples during aerobic storage at 10 °C, which is in agreement with Vold, Holck, Wasteson, and Nissen (2000), who noted that the presence of a beef microflora inhibited the growth of *E. coli* O157:H7 in beef mince aerobically stored at 12 °C. However, such interactions between pathogens and the wider beef microflora, may be influenced by other physical and/or environmental factors, as Berry and Koohmaraie (2001) reported no such effects on non-minced beef surfaces.

A number of studies have examined survival and/or growth of *E. coli* O157:H7 and other pathogens on excised/cut surfaces (Kinsella et al., 2008; Logue, Sheridan, & Harrington, 2004; Nissen et al., 2001) but much less is known about the survival and/or growth of this pathogen on uncut surfaces, i.e. surfaces with intact

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membranes. Studies of *E. coli* inoculated onto beef carcass surfaces have demonstrated that growth and survival rates are affected by the nature of the inoculated site (Kinsella, 2008; Prendergast and Sheridan, unpublished). Thus, during holding at a range of chill temperatures, some counts on the cut surface of the neck increased (while the rest remained unchanged) but on the brisket, some counts were reduced (while the rest remained unchanged). On the rump, all counts were reduced, frequently to almost zero. Overall, the results of such studies highlight the need for a more comprehensive understanding of the physical and biological factors modulating bacterial survival and growth on beef carcasses and derived products, in the development of accurate models of the growth of *E. coli* O157 (and other pathogens) in these complex food environments.

The present study examined the growth/survival of *E. coli* O157:H7 and a potential index organism (generic *E. coli*) on excised irradiated and non-irradiated beef neck, brisket and rump surfaces, and compared the growth of these organisms on these surfaces, with their growth in broth systems.

2. Materials and methods

2.1. Slaughter and inoculation sites

The study used an experimental abattoir at Ashtown Food Research Centre (AFRC), designed for low-throughput slaughter and processing of cattle, sheep and pigs. Six grass fed Hereford × Fresian heifers (⩽24 months) were processed under normal commercial slaughter conditions, i.e. lairage, stunning, exsanguination, dehiding, and evisceration, followed by carcass splitting, weighing and washing, to produce carcasses with a mean weight of 122.5 kg (range of 110.0–131.5 kg). Three sites on each carcass were identified as follows: (i) neck, (ii) brisket and (iii) rump. A fibrous membrane of connective tissue or fascia covers the latter two sites, while the selected neck site was the lean surface exposed during slaughter (i.e. not covered by fascia). Two animals were slaughtered per day, with each day representing a complete treatment. The treatment was repeated on three separate days.

2.2. Production of beef samples

Immediately after carcass washing, surface samples (approximately 300 cm^2, and 5 mm deep) were aseptically excised from each selected site, placed in sterile stomacher bags and transported to the on-site laboratory. From these surface samples cylindrical meat pieces (approximate diameter 2.5 cm^2) were aseptically excised using sterile coring punches. On each occasion, 26 × 2.5 cm^2 samples were excised from each of the three carcass sites.

Samples were placed in separate 12.5 × 7.7 cm Cryovac BB4 bags (Sealed Air Cryovac**,** Cambridge, UK), vacuum packed using a Vac Star, model S220 (Sugiez, Switzerland) and chilled to 4 °C within 1 h.

2.3. Sample irradiation

Vacuum packed samples (486) were transported under refrigeration (<10 °C) to the Agrifood Biosciences Institute, Northern Ireland, and irradiated at 4 °C (±1 °C) to a target dose of 5.0 kGy, using a cobalt 60 source (Gammabeam 650, Nordion International Inc., Kanata, Canada) at a dose rate of 5.33 kGy h^{-1}. Irradiation dose was monitored and confirmed using four red perspex dosimeters (Type 4034AN, Harwell Dosimeters Ltd., Harwell, UK) placed throughout the packed meat pieces. Used dosimeters were spectrophotometrically examined (640 nm), and doses calculated using calibration graphs provided by the National Physical Laboratory (Teddington, UK). The sample preparation process, i.e. excision, vacuum packaging, dispatch, irradiation, and return was carried out under refrigeration conditions within 72 h. Returned, irradiated samples were stored at 0 °C for no more than 24 h.

Immediately prior to inoculation, six irradiated samples were randomly selected from each sample type, placed in 30 ml BHI at 25 °C for 18 h, plated onto Oxoid Plate Count Agar (PCA) (Oxoid, Basingstoke, UK), incubated at 30 °C for 3 days, and examined to confirm sterility.

2.4. Inoculum preparation

Six bovine isolates of *E. coli* O157:H7, each of which carried the *hlyA* and *eaeA* genes were obtained from the AFRC's culture collection. Four of the isolates carried the *vt*1 and *vt*2 genes, while one each carried either the *vt*1 or *vt*2 gene only. All six isolates were made resistant to 50 μg ml^{-1} nalidixic acid (Sigma–Aldrich, St. Louis, US) and 1000 μg ml^{-1} streptomycin sulphate (Sigma–Aldrich) by the method of Park (1978). A single non-pathogenic bovine hide isolate of *E. coli*, was obtained from the AFRC culture collection. In a separate experiment, a six isolate cocktail of non-pathogenic *E. coli* was prepared to determine if there was a difference in the growth kinetics between the single isolate and a cocktail of strains.

The pathogenic and non-pathogenic isolates were individually cultured in Oxoid Brain Heart Infusion Broth (BHI) at 37 °C for 16–18 h. Stationary phase cells were recovered using an Eppendorf model 5403 refrigerated centrifuge at 3000*g* for 10 min at 4 °C (Eppendorf, Hamburg, Germany), washed three times in Oxoid maximum recovery diluent (MRD) by centrifugation and resuspended/diluted in MRD to give a final inoculum of approximately 5 $\log_{10}$ CFU ml^{-1}.

2.5. Meat surface inoculation

Thirteen irradiated (IR) and thirteen non-irradiated control (NIRC) samples from each carcass site were inoculated with a 5 μl inoculum containing approximately 3 $\log_{10}$ CFU of the cocktail of pathogenic *E. coli* (PEC) or of the non-pathogenic *E. coli* (NPEC) and held at room temperature for 30 min (to facilitate cell adherence). Inoculated samples (13) and uninoculated controls (13) were transferred to Aqualab cups (Labcell, Basingstoke, UK), covered with caps and placed in refrigerated incubators at 10 °C. Inoculated and uninoculated samples from each site (neck, rump and brisket) were removed after 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66 and 72 h, and examined as described below.

2.6. Temperature monitoring

Temperatures of the refrigerated incubators were monitored at three positions, top, middle and bottom every 30 min for 72 h, using Type T copper–constantan thermocouples and the data was recorded using a model 2040 Grant Squirrel data logger (Grant Instruments, Cambridge, UK). At the end of each experiment, data were downloaded to Microsoft® Office Excel 2003.

2.7. Enumeration of pathogenic (PEC) and non-pathogenic E. coli (NPEC)

Each recovered sample was pulsified for 30 s in 10 ml MRD in a sterile 100 × 150 mm stomacher bag (Seward, Norfold, UK) using a model PUL Pulsifier™ (Filtraflex, Ontario, Canada). Undamaged *E. coli* O157:H7 were enumerated by direct plating of 1 ml aliquots of the above homogenates onto Oxoid Sorbitol MacConkey Agar (SMAC), each supplemented with 50 μg ml^{-1} nalidixic acid and 1000 μg ml^{-1} streptomycin sulphate.