The survival characteristics of *Escherichia coli* O157:H7 and transfer from the contaminated hide to the carcass during slaughter

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Background

Cattle, with an incidence of 2 to 4 % (Chapman *et al.*, 1993; Faith *et al.*, 1996), are the primary source of *E.coli* O157:H7. Once ingested, the organism persists in the rumen and colon of the animal, contaminating the faeces (Brown *et al.*, 1997), which then acts as a vehicle in the horizontal transmission to previously uninfected animals (Hancock *et al.*, 1994; Faith *et al.*, 1996) and from cow to human.

The survival characteristics of *E.coli* O157:H7 in the environment and an investigation of the transfer of *E.coli* O157:H7 from the contaminated hide to the carcass during slaughter are reported in this paper.

Objectives

- To investigate the survival characteristics of *E.coli* O157:H7 under laboratory and environmental conditions.
- To examine the transfer of this pathogen from the contaminated hide to the carcass during slaughter.

Methods

One bead of *E.coli* O157:H7 (NCTC 12900) was aseptically transferred to 30ml BHI broth and incubated at 37°C for 24h. From this culture, 7.0ml was transferred to 700ml fresh BHI and incubated for a further 18h at the same temperature. The cells were then recovered by centrifugation at 3019g (Eppendorf Centrifuge 5403), washed three times in Maximum Recovery Diluent (MRD, 0.85g sodium chloride and 1.0g bacteriological peptone per litre of distilled water) and resuspended in 25ml MRD. Bovine faeces was collected immediately after excretion by the animal and 500ml was mixed with the 25ml of bacterial suspension to give an initial inoculation level of approximately 8 $\log_{10}$ cfu/ml. From this, 100ml was transferred to each of 2 closed sterile plastic containers, one of which was stored in the laboratory at 10°C, while the other was placed outside in the field. Adjacent to the latter, 100ml of the inoculated faeces was deposited on the grass and exposed to Irish weather conditions from mid January to mid April. Finally, 200ml of the inoculated faeces was spread on the rump area (approximately 800cm^2) of each of 10 heifers, 24 hours before slaughter.

Surviving *E.coli* O157:H7 cells were enumerated on Sorbitol MacConkey Agar (SMAC, Oxoid) and Tryptic Soya Agar (TSA, Oxoid) using the non-selective overlay recovery technique. The former were incubated at 37°C for 48h, while the TSA plates were stored at 37°C for 2h, to allow injured cells to recover, prior to overpouring with SMAC and incubation for a further 48h at the same temperature. Low numbers of *E.coli* O157:H7 still present in the soil after the faeces exposed on the grass had been washed away were detected using an enrichment procedure. Approximately 1.0g of the soil was incubated in 9.0ml of E.E. broth at 37°C for 24h. Any *E.coli* O157:H7 present were then detected using both the selective (SMAC) and the non-selective (TSA) techniques.

All implements used during slaughter were swabbed using a damp sterile cellulose sponge which was subsequently homogenised in 200ml MRD using a Colworth stomacher. The carcass was divided into sections according to Lasta *et al.* (1990) and a known area of the outside of the carcass was swabbed using a damp sterile cellulose sponge and homogenised in 200ml of MRD as before.

Both experiments were done in duplicate and repeated 5 times.

Results and Discussion

The opportunity for transfer of *E.coli* O157:H7 from infected to uninfected animals depends on the survival characteristics in bovine faeces under different environmental conditions. Studies by Maule (1995) on the survival of *E.coli* O157:H7 in different laboratory-scale ecosystems showed that bovine faeces was a relatively favourable environment for the survival of this pathogen. In our study the initial inoculum levels of 8.28 $\log_{10}$ cfu/g (selective) and 8.15 $\log_{10}$ cfu/g (non-selective) decreased to 3.18 $\log_{10}$ cfu/g and 2.57 $\log_{10}$ cfu/g, respectively, in faecal samples stored at 10°C in the laboratory for 99 days (fig. 1). Similar survival rates were observed for samples stored in sealed containers outside in the field where *E.coli* O157:H7 levels decreased by approximately 5.0 $\log_{10}$ cfu/g over the same time period. Interestingly, *E.coli* O157:H7 levels decreased by a similar amount, from 7.85 $\log_{10}$ cfu/g (selective) and 7.47 $\log_{10}$ cfu/g (non-selective) to 2.96 $\log_{10}$ cfu/g and 3.02 $\log_{10}$ cfu/g, respectively, in the bovine faeces exposed on grass after 50 days. Despite the outdoor temperature fluctuating from -6.5 °C to 19.6 °C, the organism was detected in 70% of samples after 85 days and in 20% of samples after 99 days using an enrichment technique. One of the five nalidixic strains of *E.coli* O157:H7, used in a similar study by Wang *et al.* (1996) survived in bovine faeces for up to 49 days at 37°C and for up to 56 days at 22°C while all five strains survived for up to 70 days at 5°C. All of this research suggests that *E.coli* O157:H7 can survive for several months on pasture land allowing the possibility of ingestion by other, previously uninfected, cattle.

Chapman *et al.* (1993), in an abattoir based study, found that 30% of carcasses from animals which had previously been found to be positive for *E.coli* O157 were contaminated with the organism after slaughter. In our study, with an initial inoculum of approximately 8 $\log_{10}$ *E.coli* O157:H7/g of faeces on the hide, the carcass contamination rate was 100%. However the pathogen was not evenly spread as higher levels of the organism (up to 3.36 $\log_{10}$ cfu/cm^2) were detected on the rump area of the carcass (Table 1). Interestingly, Gill *et al.* (1995) reported a similar result. The butchers hands were also heavily contaminated post slaughter as were most of the tools used during dressing operations. The chine saw (used for cutting the back bone) and small brisket saw both showed levels of contamination between $\log_{10}$ 2.35 cfu/ml and $\log_{10}$ 3.21 cfu/ml. The knives was also contaminated, but to a lesser degree, perhaps reflecting the smaller surface area of this tool. The flare was an exception, however, as it remained uncontaminated or

showed a relatively low level of contamination (-0.65 log_{10} cfu/ml). When it is considered that the infective dosage for *E.coli* O157:H7 might be less than log_{10} 0.3 cfu/g (Bolton *et al.*, 1996), the levels of *E.coli* O157:H7 detected on the carcass during this study should give cause for concern.

Conclusions

- *E.coli* O157:H7 can survive for relatively long periods of time in the environment and farm practices such as the spreading of faeces based fertilisers may facilitate the horizontal spread of this pathogen.
- Animals must be thoroughly cleaned preslaughter to ensure faecal matter does not act as a vehicle of transmission in the spread of *E.coli* O157:H7 to the carcass during slaughter.

Pertinent Literature

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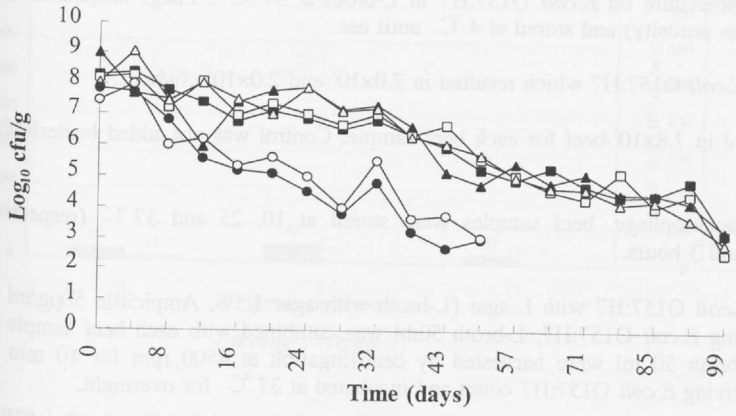

Figure 1. Survival of *E.coli* O157:H7 at 10°C in the laboratory as determined on selective (■) and non-selective (□) media, outside in field containers as determined on selective (▲) and non-selective (Δ) media and on the grass as determined on selective (●) an non-selective(○) media.

Table 1. The levels of *E.coli* O157:H7 on the carcass and in the abattoir post-slaughter

Site	Carcass Left triangle	Carcass Right triangle	Carcass Left rectangle	Carcass Right rectangle	Hands	Chine saw	Small brisket saw	Knife	Flare
	Log_{10} cfu/cm^2				Log_{10} cfu/ml				
SMAC	3.06	3.36	-0.34	1.52	3.07	3.2	3.21	1.43	0
TSA	2.57	2.35	-0.42	1.54	4.28	2.35	2.46	2.41	-0.65