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Is the prevalence and shedding concentrations of *E. coli* O157 in beef cattle in Scotland seasonal?

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Abstract

The prevalence of *Escherichia coli* O157 in Scottish beef cattle at abattoir was found to be greater during the cooler months [11.2% (95% CI, 8.4–13.9%)] compared to the warmer months [7.5% (95% CI, 5.4–9.6%)]; the reverse of seasonality of human infections. However, high shedding beef cattle (excreting $> 10^4$ g^{-1}) appear to shed greater concentrations of *E. coli* O157 in the warmer months which may partly explain increased human infection seasonality at this time.

Keywords: E. coli O157; Cattle; Pathogen prevalence; Pathogen concentration; Seasonality of infection

1. Introduction

Escherichia coli O157 is a relatively rare but nevertheless serious gastrointestinal pathogen with sequelae ranging from watery to bloody diarrheoea, vomiting, haemolytic uraemic syndrome and in some cases death. Human infection can occur via a range of routes including foodborne, waterborne, direct or indirect contact with animals and their faeces as well as person to person spread. There is a strong worldwide seasonality of infection in humans peaking in the warmer summer months and dipping during the cooler winter months [1,2].

The main reservoir of *E. coli* O157 is considered to be ruminants and in particular cattle which have a highest prevalence in the United States during the warmer summer months [3] and which has been used to partly explain increased human infection at this time [4]. In Scotland however, prevalence studies [5] in beef suckler cattle destined for food have shown that herd level prevalence is greatest in animals that are housed (i.e. during the cooler months) and that individual animal prevalence is significantly greater in housed animals compared to those on pasture [6].

We have previously reported [7] a study of Scottish beef cattle at slaughter during May–July 2002 where individual animal prevalence was 7.5% (95% confidence interval (CI), 5.4–9.6%) and the group prevalence was 40.4% (95% CI, 27.7–53.2%). Of the 44 infected animals detected, 9% were high shedders that contained *E. coli* O157 at concentrations of $>10^4$ CFU g^{-1}. These 9% represented >96% of the total *E. coli* O157 produced by all animals tested. The aims of this study were: (i) to estimate prevalence and concentrations of *E. coli* O157 in beef cattle during the winter period, (ii) to compare these data with those obtained in the previous summer investigation [7] and (iii) to determine whether seasonality of shedding in beef cattle reflects human infection rates.

2. Materials and methods

2.1. Sample collection

Faecal samples were collected from the process line at the same [7] abattoir (sourcing animals from the whole

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of Scotland) during weeks beginning January 13th–March 3rd 2003 ($n = 511$) by rectum retrieval. Samples were placed in sterile plastic bags, stored in a cool box and transported to the laboratory within 3 h.

2.2. Isolation of E. coli O157

Samples were analysed for *E. coli* O157 by enrichment followed by immunomagnetic separation (IMS). Each faecal sample (25 g) was homogenised with 225 ml buffered peptone water (BPW, Oxoid CM509) supplemented with vancomycin 8 mg l^{-1} and incubated at 42 °C for 6 h. To determine the presence or absence of *E. coli* O157, 1 ml of the enriched sample was analysed by IMS (KingFisher mL, Thermo Life Sciences, Basingstoke, UK) using 0.02 ml Captivate™ *E. coli* O157 immunomagnetic beads (International Diagnostic Group, Bury, UK). After IMS, the beads were washed three times (phosphate-buffered saline (PBS) + Tween 20) and re-suspended in 0.1 ml (same buffer) and spread equally on two sorbitol MacConkey agar plates (SMAC, Oxoid CM813) supplemented with cefixime, 0.05 mg l^{-1}, and potassium tellurite, 2.5 mg l^{-1} (CT-SMAC, Mast Diagnostics, Merseyside, UK) and incubated at 37 °C for 18–24 h. Presumptive *E. coli* O157 colonies (non-sorbitol fermenting) were confirmed by latex agglutination (Oxoid DR620). Positive isolates were further confirmed biochemically by the production of indole from tryptone water at 44 °C and genotypically (see below). The remainder of each faecal specimen was stored at 4 °C for further analysis.

2.3. Enumeration of E. coli O157

Enumeration of IMS positive *E. coli* O157 faecal samples was performed (the day following positive confirmation) by serially diluting (10^{-1}–10^{-4}) a further 25 g of faeces with PBS. From each dilution, 0.1 ml was spread onto Harlequin™ SMAC BCIG (International Diagnostic Group, Bury, UK) supplemented with cefixime and tellurite (as above) and CTSMAC agars. Plates were incubated at 37 °C for 18–24 h and presumptive colonies (five randomly selected when more than five were present on the plate) were confirmed *E. coli* O157 by latex agglutination and biochemically, as above and enumerated manually.

2.4. Identification of virulence markers

Detection of virulence markers (vt_1, vt_2 and *eaeA* genes) in the positive isolates was determined by PCR [8]. The amplification products were separated on 1.5% agarose gel in 0.5 tris-borate–EDTA buffer and visualised under UV using a 100 bp ladder as a standard (Amersham Biosciences, Bucks, UK). Expected product sizes were vt_1, 282 bp; vt_2, 164 bp and *eaeA* 410 bp.

2.5. Human incidence data

Data on weekly rates of *E. coli* O157 infection in humans in Scotland during 1998–2002 were obtained from the Scottish Centre for Infection and Environmental Health [9].

2.6. Statistical analysis

Microsoft Excel was used to determine 95% binomial confidence intervals of *E. coli* O157 prevalence in faecal carriage at the individual and group level of infected animals. A χ^2 test was used to compare prevalence data in cattle for the current winter (January–March) study to that found in the previous summer (May–July) study. A two sample *t*-test was used to compare human infection rates between the warmer months and the cooler months.

3. Results

3.1. Prevalence and concentration of E. coli O157

The range of concentrations of *E. coli* O157 shed in this study and those in our previous summer study are presented in Table 1. The prevalence of individual animals shedding *E. coli* O157 in the cooler months was estimated to be 11.2% (95% CI, 8.4–13.9%) which was greater than that we reported in the warmer months 7.5% (95% CI, 5.4–9.6%) (Table 2). Although the confidence intervals overlap, this seasonal trend was found to be different by the χ^2 test at the $p = 0.035$ level. The prevalence of each finishing group having at least one animal positive was 33.7% (95% CI, 24.2–43.2%) in the winter and 40.4% in the summer (95% CI, 27.7–53.2%) This seasonal difference was found not to be significant ($p = 0.41$) by the χ^2-test.

Fig. 1 shows the frequency of the distribution of concentrations shed by the animals in both studies. The number of high shedding individuals (defined here as $>10^4$ g^{-1}) was found to be similar for both the warmer

Table 1
Range of *E. coli* O157 concentrations in the summer and winter abattoir cattle faecal samples

E. coli O157 (CFU g^{-1})	Number of cattle	
	Summer	Winter
Negative	545	454
$<10^2$ (g^{-1})	27	44
10^2–10^3	6	7
10^3–10^4	7	3
10^4–10^5	2	3
10^5–10^6	2	0
Total positive	44	57

Table 2
Seasonality of prevalence and shedding concentrations of *E. coli* O157 in beef cattle and number of reported cases of *E. coli* O157 in the human population

	Cooler months (13th January – 9th March)	Warmer months (20th May – 21st July)
Prevalence in beef cattle (%)	11.2	7.5
Average concentration shed in beef cattle (CFU g^{-1})	330	1932
Human cases in Scotland (average number of cases per year during the 8-week period)	13	53

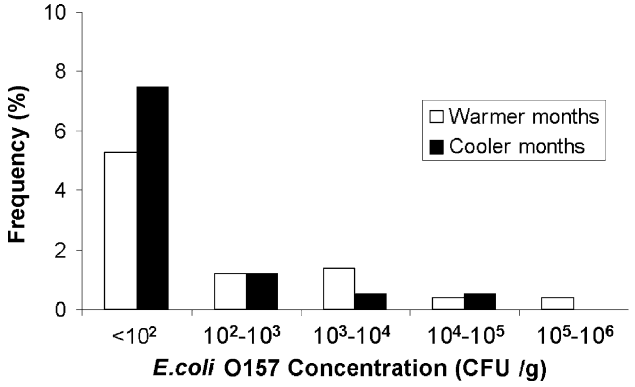

Fig. 1. Concentration of *E. coli* O157 shed by beef cattle at slaughter.

and cooler months (0.7% and 0.6%, respectively). During the warmer months the high shedders appear to shed higher concentrations resulting in a sixfold increase in numbers shed at this time of year (Table 2).

3.2. Virulence markers

The majority (98%) of strains in this winter study were potentially pathogenic to humans having both the attaching gene *eaeA* and either vt_1 and/or vt_2 genes (Table 3).

Table 3
Presence of *E. coli* O157 virulence markers in summer and winter studies

Virulence genes			Summer	Winter
EaeA	vt_1	vt_2	Jan–Mar	May–July
+	+	+	11.4%	14%
+	–	+	77.3%	84%
–	–	–	0%	1.8%
–	–	+	11.4%	0%

3.3. Human infection data

When human infection rates were compared between warmer and cooler months, there was strong evidence ($p = 0.0012$) to show summer predominated (with on average a fourfold increase, Table 2).

4. Discussion

Results presented here, and those of Synge 2000 [6] show prevalence of *E. coli* O157 in Scottish beef cattle to be greater in the winter compared to summer. In a third Scottish study [5], an autumnal rise (peaking in November) in the shedding of *E. coli* O157 was observed in beef herds having at least one positive animal; May and June having the lowest prevalence. These data oppose those from N. America [10,11], Italy [12] and Ireland [13] where studies report greatest prevalence during the warmer months. Our observations may be due to the fact that cattle are housed during winter in Scotland and therefore in close proximity to one another facilitating animal to animal cross contamination. Synge et al. [5] showed housing of animals was significantly ($p < 0.001$) linked to shedding of *E. coli* O157 while results from a Swedish study [14] suggested that calves on pasture may be less exposed to *E. coli* O157 than housed animals. Paiba (personal communication) also found housing in the UK to be significant risk factor in shedding of *E. coli* O157 in beef cattle.

The prevalence data in our studies indicate the seasonality of *E. coli* O157 infections in the human and animal populations appear to be contradictory. Cattle prevalence data at both the individual and group level may be a relatively poor indicator of the size of the reservoir of *E. coli* O157. The inclusion of concentration data may give a better estimate of the quantity of the pathogen shed by the beef cattle population. Here we have shown a sixfold rise in concentration of *E. coli* O157 shed in the summer compared with the winter and this trend follows that of human infections. However, we must treat this result with caution because this difference in shedding concentration is due to a few high shedding cattle and as such, further studies are required to determine the significance of this result. Investigations into the presence of *E. coli* O157 in retail foods have also shown a greater prevalence during summer where in a 12 month study Chapman et al. [15] found 82% of retail meats testing positive for *E. coli* O157 were collected between May and September and in US [4] workers found ground beef samples were three times as likely to contain *E. coli* O157 in the period June–September as at other times. However, it must be pointed out that concentrations of *E. coli* O157 were not calculated. Other factors which must also be considered that may contribute to peak human infection rates in the

summer include: increased likelihood of contact with farm animals and their faeces (i.e. more visits to the countryside in warm weather and these will include contamination from beef cattle sources but also dairy cattle and sheep); increased ambient temperature thus potentially enabling growth of the organisms on carcasses and in faeces [16] and changing of eating habits (e.g. more barbecues in the summer). These factors are all sensitive to the reservoir of *E. coli* O157 and animals shedding high concentrations contribute the most to the reservoir as well as pose the greatest risk to the human population.

Virulence profiles of isolates found in this investigation are comparable to both our previous summer study and clinical *E. coli* O157 isolates in Scotland where in 2002, 81% (Scottish *E. coli* O157 Reference Laboratory, personal communication) were vt_1 negative, vt_2 positive. This is further evidence that beef cattle are a major source of human *E. coli* O157 infections.

Quantitative microbiological risk assessments (QMRA) have been developed for both foodborne [4] and environmental [17] pathways of infection. Combining these type of risk models parameterised with seasonal data (e.g. concentration and prevalence of *E. coli* O157 in farm animals) should yield the seasonality of human infection (Table 2). This methodology will help elucidate the relative importance of the various infection pathways and would offer the advantage of evaluating potential risk mitigation strategies *in silico*.

5. Conclusions

Infections from *E. coli* O157 in Scotland peak in the warmer months whilst cattle prevalence is greater during the cooler months when they are housed. During the warmer months there appears to be a greater quantity of *E. coli* O157 shed by high shedding animals. This combined with the increased human exposure to *E. coli* O157 by foodborne and environmental pathways and increased ambient temperature possibly resulting in the growth of the organism in food and environmental matrices may explain the seasonality of human infection in Scotland.

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