TECHNICAL REPORT for the Food Standards Agency Scotland

QUANTIFYING THE SEASONALITY OF *E. COLI* O157 SHEDDING (CONCENTRATION AND PREVALENCE) IN CATTLE AND ESTIMATING ITS EFFECT ON THE NUMBER OF CASES OF FOOD POISONING

Project Code S01018

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Project start date: January 2003
Project end: May 2003
Report date: May 2003

Executive summary

E. coli O157 infection exhibits strong seasonality in Scotland with approximately four times as many cases in the warmer summer months compared with the cooler winter months. The concentration and prevalence of *E. coli* O157 in cattle faeces at a local abattoir was studied from January to March 2003. The methods employed were identical to a previous study that had been carried out during May to July 2002. The aim was to compare the results between the warmer months when cattle are mainly at pasture and the cooler months when they are generally housed to try and find an explanation for the seasonality of infection in humans.

The prevalence of *E. coli* O157 in cattle faeces during the warmer months was found to be 7.5% which was significantly less than the 11.2% found during the cooler months (P=0.05). This is **opposite** to the seasonality of human infection. Both the warmer month study and the cooler month study identified virtually the same numbers of animals shedding high loads (>10^4 CFU/g) of *E. coli* O157, 0.7% and 0.6% respectively. However, during the warmer months the high shedders appear to shed higher concentrations resulting in an 8-fold increase in numbers excreted at this time of year. This may partly explain the seasonality of infection in humans.

The vast majority of strains isolated during the studies were potentially pathogenic to humans. Most (82%) were vt_1 negative, vt_2 positive, which is comparable to the ratios of clinical *E. coli* O157 isolates in Scotland, where in 2002, 81% were vt_1 negative, vt_2 positive. This is further evidence that cattle are a potential source of human *E. coli* O157 infection. Previous and ongoing studies determining only the prevalence of *E. coli* O157 in a group of animals may be a poor indicator of the total number of organisms shed by the group and may be a poor indicator of the risk of human infection. We propose that a far better indicator is knowledge of both the prevalence and the numbers of organisms shed – in particular from high shedding individuals.

Some method of removing these high shedding animals from the food chain or causing them to shed less *E. coli* O157 may reduce the risk of food poisoning. However, it is important to determine the duration of the high shedding (e.g. is it a day or is it a couple of

weeks) as this would determine the feasibility of testing cattle at the farm prior to being moved to the abattoir.

Despite significant numbers of cattle shedding *E. coli* O157 in their faeces and there being high shedders the actual number of humans being infected by *E. coli* O157 in Scotland is low (approximately 200-300 cases per year). This indicates that hygiene measures at abattoir and along the food chain must be operating well. However, this view must be treated with caution because a breakdown of hygiene/processing standards in the food chain could potentially lead to another large food outbreak of *E.coli* O157.

Introduction

Escherichia coli O157 is a relatively rare but significant gastrointestinal pathogen with sequelae ranging from watery to bloody diarrhoea, vomiting, haemolytic uraemic syndrome and in some cases death (Griffin and Tauxe, 1991). 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 (Smith et al., 1998). There is a strong worldwide seasonality of infection in humans peaking in the warmer summer months and dipping during the cooler winter months (Wallace et al., 2000). The main reservoir of *E. coli* O157 is considered to be cattle (Russell et al., 2000) which have a highest prevalence in the United States during the warmer summer months (e.g. for feedlot cattle which are generally kept outside throughout the year -Hancock et al., 2001) and which has been used to partly explain increased human infection at this time (The National Academies, 2002). However, in Scotland, prevalence studies in fattening cattle destined for food have shown that there is no peak of prevalence during the warmer months and that individual animal prevalence is significantly greater in housed animals compared to those on pasture (Ternent, 2002). Since most animals are housed in the cooler months and are on pasture during the warmer months it appears that the seasonality of prevalence in the cattle population in Scotland is opposite to that of human infections (Figure 1).

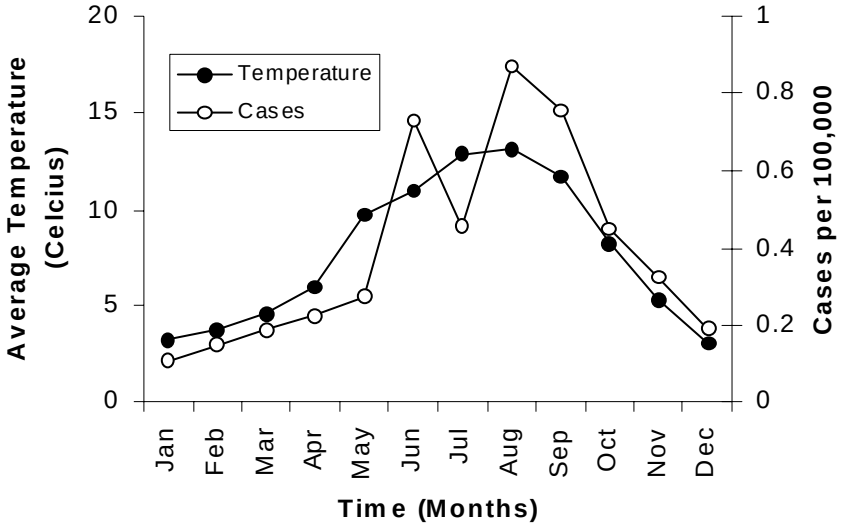

Figure 1. Average monthly temperature (1998-2002) (Met Office, 2003) and *E. coli* O157 human infections (SCIEH) in Scotland (1998-2002).

A previous study performed by Aberdeen University (Omisakin et al., 2003) determined the prevalence and concentration of *E. coli* O157 being shed by cattle at slaughter during some of the warmer months of the year (May to July 2002) when cattle are generally at pasture. The publication (Omisakin et al., 2003) describing this work is appended to the back of this report. The study found that the prevalence was 7.5% (44/589) at the individual animal level and 40.4% at the group level. Of the 44 infected animals detected, 9% were high shedders that contained concentrations of $>10^4$ CFU g^{-1}. These 4 animals represented > 96% of the total *E. coli* O157 produced by all animals tested.

The current study was a repeat of the previous one but this time conducted during the cooler months of the year. The aim being to determine if a change in prevalence and concentration of shedding of *E. coli* O157 by cattle between the cooler and warmer months could help explain the seasonality of human infection. We had two opposing hypotheses which we wished to test:

(i) Farm animals (e.g. cattle) shed higher concentrations of *E. coli* O157 in the summer months. This leads to higher concentrations contaminating carcasses thus leading to a higher incidence of food poisoning in the summer months.

(ii) Farm animals do not shed higher concentrations in the summer months. The reason for increased foodborne infection rates in the summer must be due to higher ambient temperatures. The higher temperature enables growth on carcasses/beef products thus increasing the probability of human infection.

If hypothesis (i) is true, then it becomes more important to control likely faecal contamination of the carcass at the abattoir during the summer months and to remove high shedding animals from the food chain.
If hypothesis (ii) is true then it becomes more important to ensure adequate temperature control from abattoir to consumption to prevent growth of *E. coli* O157 in food products.

In testing these hypotheses the 3 FSAS project objectives will be completed. These are:

1. Measure the prevalence of *E. coli* O157 in faeces in abattoir cattle,
2. Measure the concentration of *E. coli* O157 in faeces in abattoir cattle,

3. Compare the prevalence and concentration of *E. coli* O157 in abattoir cattle during January-March 2003 with previous data collected in May-July 2002 (Omasakin et al., 2003).

Materials and Methods

Sampling

Faecal samples were collected from a local abattoir between January 13th to March 3rd 2003 (n=511). This was done on the process line of the factory by rectum retrieval and extracting the faecal contents. Samples were placed in sterile plastic bags, stored in a cool box and transported to the laboratory within 3 h.

Isolation method for *E. coli* O157

Samples were analysed for *E. coli* O157 by enrichment followed by immunomagnetic separation (IMS) (Ogden et al., 2001). Each faecal sample (25 g) was homogenised with 225 ml buffered peptone water (BPW, Oxoid CM509) supplemented with vancomycin 8 mg/l 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) and potassium tellurite (2.5 mg/l) [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.

Determine the concentration of *E. coli* O157

Enumeration of IMS positive *E. coli* O157 faecal samples was performed 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 >five were present on the plate) were confirmed *E. coli* O157 by latex agglutination and biochemically, as above and enumerated manually.

Identification of virulence markers

Detection of virulence markers (vt_1, vt_2 and *eaeA* genes) in the positive isolates was determined by PCR (Lin et al., 1993). The amplification products were separated on 1.5% agarose gel in 0.5 tris-borate-EDTA buffer and visualised under UV using a 1000 base pair ladder as a standard (Amersham Biosciences, Bucks, UK). Expected product sizes were vt_1, 282 bp; vt_2, 164 bp and *eaeA* 410 bp.

Results and Discussion

Table 1 details animals, which were found to be positive, the concentrations of *E. coli* O157 shed by these animals and the detection of the presence/absence of virulence genes determined by PCR. This table completes FSAS objectives 1 and 2. The results/discussion for objective 3 are given in the following paragraphs.

Table 1 Concentration and presence of virulence genes for *E. coli* O157 isolates from winter study.

Week number	Group (Farm) number[**]	Lab number[*]	Concentration of +ve sample	*vt1*	*vt2*	*eaeA*
1	1	8	<100/g	-	+	+
1	7	38	<100/g	+	+	+
1	8	40	<100/g	+	+	+
1	8	42	1.0×10^2/g	+	+	+
1	8	43	<100/g	+	+	+
1	8	44	<100/g	+	+	+
1	8	45	<100/g	+	+	+
1	8	46	<100/g	+	+	+
1	12	66	<100/g – atoxigenic	-	-	-
2	1	9	<100/g	-	+	+
2	4	24	<100/g	-	+	+
2	8	32	<100/g	+	+	+
2	12	48	<100/g	-	+	+
2	13	62	1.8×10^4 /g	-	+	+
2	13	63	<100/g	-	+	+
2	13	64	<100/g	-	+	+
2	16	83	<100/g	-	+	+
2	17	95	<100/g	-	+	+
2	18	97	<100/g	-	+	+
2	18	100	7.9×10^3/g	-	+	+
2	18	101	1.8×10^3/g	-	+	+
2	18	102	8.5×10^2/g	-	+	+
2	18	107	1.0×10^2/g	-	+	+
2	18	108	7.5×10^2/g	-	+	+
2	18	109	<100/g	-	+	+
2	19	116	3.0×10^2/g	-	+	+
3	9	37	<100/g	-	+	+
3	9	41	<100/g	-	+	+
3	9	42	<100/g	-	+	+
3	10	44	<100/g	-	+	+
3	12	70	8.3×10^3/g	-	+	+
3	12	71	<100/g	-	+	+
4	3	62	<100/g	-	+	+
4	6	86	<100/g	-	+	+

[*] The laboratory number is the number of the particular sample for a given week.
[**]The group number (farm number) is the group number for a particular sample for a given week.

Table 1. continued

Week	Group (Farm) number**	Lab number *	Concentration of +ve sample	*vt1*	*vt2*	*eaeA*
5		No positives				
6	2	2	<100/g	-	+	+
6	2	4	<100/g	-	+	+
6	2	5	<100/g	-	+	+
6	4	21	<100/g	-	+	+
6	4	22	<100/g	-	+	+
6	6	29	2.0×10^2/g	-	+	+
6	7	33	<100/g	-	+	+
6	7	34	1.0×10^2/g	-	+	+
6	7	35	<100/g	-	+	+
6	9	41	<100/g	-	+	+
6	11	56	<100/g	-	+	+
6	12	62	<100/g	-	+	+
6	14	74	<100/g	-	+	+
7	3	11	<100/g	-	+	+
7	4	19	<100/g	-	+	+
7	5	20	3.9×10^4/g	-	+	+
7	5	21	<100/g	-	+	+
7	5	22	<100/g	-	+	+
7	9	55	<100/g	-	+	+
7	9	59	1.7×10^4/g	-	+	+
7	9	60	<100/g	-	+	+
7	10	62	<100/g	-	+	+
8	1	3	<100/g	-	+	+

* The laboratory number is the number of the particular sample for a given week.
** The group number (farm number) is the group number for a particular sample for a given week.

The prevalence of animals shedding *E. coli* O157 in this study, conducted over the cooler months, was 11.2% (57/511) which was greater than the 7.5% (44/589) found in the warmer months. This seasonal difference was found to be significant (p=0.05) by the χ^2 test (Lewis and Traill, 1999) and these data are in general agreement with the elevated prevalence for housed animals in the winter months found by Ternent (2002). Ternent reported that samples from 11% of housed cattle and 5% of cattle at pasture were positive). A two sample t-test (Lewis and Traill, 1999) was performed to determine if infection rates in the human population (Figure 1) were greater during the warmer months (June & July)

compared with the cooler months (January-March). There was strong evidence (p=0.002) to show that this was in fact the case with on average a four-fold increase of human infections (approximately 7.5 cases per month in January-March compared with 30 cases per month June & July) in the warmer months. Hence, these data indicate that the seasonality of infections in the human and cattle populations appear to be contradictory.

The majority of animals with *vt1* (8/9) were found in week one and closer examination of the data revealed that all but one of the eight animals were from the same farm. It is likely that the same strain of *E. coli* O157 containing all three virulence genes had been spread between animals sent to slaughter on that date. Figure 2 shows that during the colder months there appear to be more animals shedding low concentrations of *E. coli* O157 (<100 /g) than during the summer months (7.5% compared with 5.3%). This may be due to the fact that housed animals are in closer vicinity to each other, contaminating each other but not necessarily colonising the gut. The prevalence of each finishing group having at least one animal positive was 40.4% in the summer and 33.7% in the winter. This seasonal difference was found not to be significant (p=0.41) by the χ^2 test.

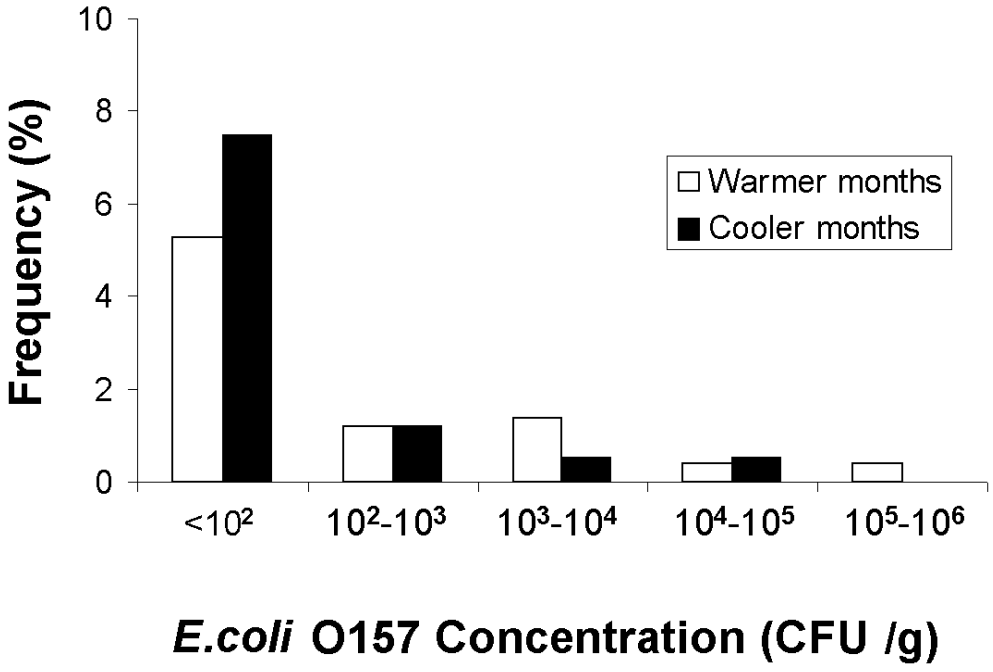

Figure 2. Concentration of *E. coli* O157 shed by cattle at abattoir.

The number of high shedding individuals (defined here as $>10^4$ /g) is approximately the same for both the warmer and cooler months (0.7% (4/589) and 0.6% (3/511) respectively). However, during the warmer months the high shedders appear to shed higher concentrations resulting in an 8-fold increase in numbers shed at this time of year. This could partly explain the seasonality of human cases but more data are required to demonstrate the significance of these results. Other factors which may contribute to increased human infection rates in the summer include: increased likelihood of contact between farm animals and their faeces; increased ambient temperature thus potentially enabling growth of the organisms on carcasses and in faeces (Wang et al., 1996) and changing of eating habits (e.g. more barbecues in the summer). All of these factors are 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.

Some method of removing these high shedding animals from the food chain or causing them to shed less *E. coli* O157 may reduce the risk of food poisoning. However, it is important to determine the duration of the high shedding (e.g. is it a day or is it a couple of weeks) as this would determine the feasibility of testing cattle at the farm prior to being moved to the abattoir.

Previous and ongoing (e.g. Hancock et al., 2001; Ternent 2002 etc.) studies which only determine the prevalence of *E. coli* O157 in a group of animals may be a poor indicator of the total number of organisms shed by the group and a poor indicator of the risk of human infection. A far better indicator is knowledge of both the prevalence and the numbers of organisms shed – in particular from high shedding individuals.

Recent reports (Naylor et al., 2003) have identified the rectal anal junction (RAJ) as a site of colonisation of *E. coli* O157 in the bovine host. The methodology used in this study which involved emptying the faeces from the rectum, not through the anus but in the opposite direction may have underestimated the prevalence and concentration in the animals tested. However, the prevalence obtained are similar to previous studies sampling faecal pats and it is very probable that part of the faeces sampled will have been in contact with the RAJ.

Table 2 gives a summary of the virulence genes found in the *E. coli* O157 isolated obtained during the winter and summer studies. It can be seen that for both studies that most strains were potentially pathogenic. Both the winter and summer studies were dominated by isolates (82%) which were *eae* +ve, vt_1 –ve and vt_2 +ve. This is comparable to the ratios of 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 cattle are a major source of human *E. coli* O157 infections.

Table 2. Virulence genes detected by PCR from *E. coli* O157 isolates obtained during the winter and summer studies.

Presence of virulence genes			Number of isolates summer	Number of isolates winter	Total (%)
eae	vt_1	vt_2			
+	+	+	5	8	13 (12.9)
+	-	+	34	48	82 (81.2)
-	-	-	0	1	1 (1.0)
-	-	+	5	0	5 (5.0)
Total			44	57	101 (100)

Despite significant numbers of cattle shedding *E. coli* O157 in their faeces and there being high shedders the actual number of humans being infected by *E. coli* O157 in Scotland is low (approximately 200-300 cases per year). This indicates that hygiene measures at the abattoirs and along the food chain must be operating well. However, this view must be treated with caution because a breakdown of hygiene/processing standards in the food chain could potentially lead to another large food outbreak of *E. coli* O157.

Quantitative microbiological risk assessments (QMRA) have been developed for both foodborne (Cassin et al., 1998; National Academies of Science, 2002) and environmental (Strachan et al., 2002) 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 (Figure 1). This

methodology will help elucidate the relative importance of the various infection pathways and would offer the potential of evaluating potential risk mitigation strategies *in silico*.

Conclusions

The prevalence of *E. coli* O157 in cattle was found to be higher during the winter months compared with the summer months. This is opposite to the seasonality of human infection and agrees with previous Scottish studies (Ternent, 2002). Similar numbers of high shedding animals were found in both the winter and summer studies. However, the high shedders in the summer appear to shed higher concentrations than those in the winter. This results in an 8-fold increase in the numbers of *E. coli* O157 shed in the summer compared to the winter. This could partly explain the seasonality of infection in humans. Further research is required to quantify the duration of high shedding to determine whether intervention strategies may be applied to remove these animals from the food chain and subsequently reduce the risk of food poisoning. Results from surveys of cattle quoting prevalence only should be treated with caution as this may not be a good indicator of the actual numbers of organisms shed and hence may be a poor indicator of the potential risk to the human population.

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