Prevalence of *Escherichia coli* O157 : H7 and serogroups O26, O103, O111 and O145 in sheep presented for slaughter in Scotland

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Sheep have been proposed as a source of human verocytotoxigenic *Escherichia coli* infection on a number of occasions but few prevalence studies have focused on identifying rates of carriage of these pathogens in this species. The purpose of this work was to establish the frequency of excretion of *E. coli* of serogroups O157, O26, O103, O111 and O145 in sheep presented for slaughter in Scotland and to examine their carriage of known virulence determinants. The study involved microbiological isolation of *E. coli* from 1082 sheep presented for slaughter in four Scottish abattoirs between July 2005 and June 2006. Using faecal enrichment and immunomagnetic separation, the isolation rate from these samples was 3.4 % for *E. coli* serogroup O157, 5.2 % for *E. coli* serogroup O26, 2.3 % for *E. coli* serogroup O103 and 0.1 % for *E. coli* serogroup O145. *E. coli* O111 was not isolated. In the last month of testing, which coincided with sorbitol-fermenting *E. coli* O157 (SFO157) cases in children in Scotland, all 83 recta received were screened and tested negative for SFO157 strains. The study found no verocytotoxin-positive strains amongst the *E. coli* serogroup O103 or O145 isolates. Verocytotoxin-positive strains were identified amongst isolates of *E. coli* serotypes O157 : H7 and O26 : H11. *E. coli* O157 : H7 was not isolated from samples collected between January and March, a statistically significant drop (P<0.001) in mean shedding relative to other months. There was evidence (P=0.003) of higher shedding of O157 in adults and hoggs than in lambs. *E. coli* O26 : H11 was isolated throughout the year, with a statistically significant peak in shedding in the third quarter (P=0.003). The results showed that sheep presented for slaughter in Scotland may carry strains of *E. coli*, particularly of serogroups O157 and O26, which can be presumed to have potential to cause human infection. They did not support a hypothesis that human cases of *E. coli* O157 : H7 are higher in any particular Scottish region as a direct consequence of a higher rate of faecal carriage in sheep in that region.

INTRODUCTION

*Escherichia coli* strains that produce verocytotoxins are important pathogens causing human illnesses ranging from diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome (Karmali, 1989; Riley, 1987). In the UK, North America, Japan and much of continental Europe, such disease is commonly caused by *E. coli* strains of serogroup O157 (Riley et al., 1983; Kleanthous et al., 1990; Willshaw et al., 2001; Kaper et al., 2004; Chase-Topping et al., 2008).

Variation in infection routes, including food-borne transmission and person-to-person spread, have been described, but in the UK, human infections by *E. coli* O157 are also recognized arising from direct contact with animal reservoirs (Locking et al., 2001; O’Brien et al., 2001). Examples of where infection has been specifically associated with sheep include: illness linked with lambing ewes (Allison et al., 1997); sheep faeces apparently contaminating the water supply at a campsite outbreak (Licence et al., 2001); and an outbreak at a boy-scout camp caused through contact with sheep faeces (Strachan et al., 2001). Though sheep have been proposed as sources for human infection on a number of occasions, there have been few prevalence studies of verocytotoxigenic *E. coli* in sheep. Therefore, the primary purpose of this work was to...
establish the frequency of excretion and to examine any regional or seasonal variation in the carriage of \textit{E. coli} serogroup O157 in sheep presented for slaughter in Scotland.

Elsewhere in Europe, other \textit{E. coli} serogroups, especially O26, O103, O111 and O145, which may include verocytotoxin producers, have been linked with human disease (Lopez et al., 1989; Bielaszewska et al., 1996; Boerlin et al., 1999; Elliott et al., 2001; Gerber et al., 2002; Tozzi et al., 2003; Beutin et al., 2004; Johnson et al., 2006).

The study’s secondary objective was to isolate these pathogens from sheep faeces in Scotland and to characterize the recovered \textit{E. coli} of serogroups O26, O103, O111 and O145 for possession of known virulence determinants.

**METHODS**

**Sample collection.** The study design was developed in light of practical constraints, current knowledge and expert opinion of the likely prevalence of faecal shedding of verocytotoxigenic \textit{E. coli} in sheep. The power calculations incorporated an assumption of a trimodal distribution of flock prevalences, assuming an overall national prevalence of 2\% of sheep being \textit{E. coli} O157:H7-positive with these animals distributed in 6\% of flocks that have low within flock prevalence and 2\% of flocks exhibiting a high within flock prevalence where 75\% of animals would be positive. The remaining 92\% of flocks were assumed negative. Power calculations indicated that collecting five samples from each of 250 lots would facilitate estimation of the true mean prevalence to an average tolerance of less than ±1.5\%.

Abattoirs were identified in four areas of Scotland, in order to sample from geographically dispersed sites, given the wide range in human infection rates seen in different areas of Scotland. Two abattoirs were from regions that consistently report high rates of human infection with \textit{E. coli} O157:H7 (Grampian, Dumfries and Galloway) and two were from regions where rates tend to be towards or lower than the national average (Greater Glasgow and Highland) (personal correspondence to J. C. Low).

Each abattoir was visited once over the course of each month from July 2003 until June 2006 with the order of sampling each month being randomized. Five separate and random lots of sheep for slaughter were chosen from amongst the lots submitted to each abattoir on the visit day. After killing, the recta were collected on the slaughter day and up to five randomly chosen individuals from each lot. Approximately 15 cm of the terminal rectum including the anal sphincter was taken from slaughtered sheep. In total, 1082 recta were received in that month were screened for sorbitol-fermenting \textit{E. coli} O157 where the specific IMS beads were used to inoculate both TBX and CT-SMac agar and up to ten colonies from each agar plate were tested by latex agglutination for \textit{E. coli} O157. The protocol alteration was necessary because these strains are reportedly sensitive to potassium tellurite, one of the selective agents present in CT-SMac routinely used for isolation of non-sorbitol-fermenting \textit{E. coli} O157 (Zadik et al., 1993).

Faecal samples containing presumptive \textit{E. coli} of serogroups O157 or O26 were retrospectively examined to enumerate the target organisms by limiting dilution. Briefly, 1 g faeces was diluted sequentially in 9 ml minimal recovery diluent and 100 μl aliquots were cultured directly onto CT-SMac or CT-RMac plates. Typical colonies were counted after overnight incubation at 37°C.

Cases of human infection in Scotland and England caused by sorbitol-fermenting \textit{E. coli} O157:H1− (HPS, 2006; Pollock et al., 2010) coincided with the last month of abattoir sampling. All 83 recta received in that month were screened for sorbitol-fermenting \textit{E. coli} O157 where the specific IMS beads were used to inoculate both TBX and CT-SMac agar and up to ten colonies from each agar plate were tested by latex agglutination for \textit{E. coli} O157. The protocol alteration was necessary because these strains are reportedly sensitive to potassium tellurite, one of the selective agents present in CT-SMac routinely used for isolation of non-sorbitol-fermenting \textit{E. coli} O157 (Zadik et al., 1993).

**Sample processing.** The 1 g faecal samples and swabs were suspended individually in 20 ml buffered peptone water (BPW), while the 10 g faecal samples were suspended in 225 ml BPW. The BPW suspensions were incubated at 37°C (±1°C) for 6 h and then separate 1 ml aliquots were subjected to testing by immunomagnetic separation (IMS). To capture \textit{E. coli} of serogroups O26, O103, O111, O145 or O157, antibody-specific paramagnetic beads were used and the IMS method was carried out as previously described (Jenkins et al., 2003). Suspensions of the serogroup O157 beads were subcultured onto MacConkey agar containing sorbitol, cefixime (0.05 mg l⁻¹) and tellurite (2.5 mg l⁻¹) (CT-SMac). The other serogroup-specific beads were cultured on chromocult TBX plates, with \textit{E. coli} O26 beads additionally subcultured onto rhamnose MacConkey agar supplemented with cefixime (0.05 mg l⁻¹) and tellurite (2.5 mg l⁻¹) (CT-RMac). All these media plates were incubated overnight at 37°C (±1°C).

**E. coli isolation and identification.** Sorbitol non-fermenting colonies on CT-SMac agar were subcultured onto chromocult coliform agar, incubated at 37°C (±1°C) overnight and any resulting red colonies were tested with anti-\textit{E. coli} O157 latex for agglutination. From the chromocult TBX plates, up to ten morphologically different colonies were tested with antisera for serogroups O26, O103, O111 or O145 by slide agglutination. Colonies growing on the CT-RMac agar that did not ferment rhamnose were tested for agglutination against O26 antisera. Presumptive positive isolates were subcultured onto MacConkey agar and then stored on beads at −80°C.

**PCRs for strain characterization.** Multiplex PCR was used to detect genes encoding verocytotoxin types (\textit{vtxC}, \textit{vtxB}), intimin (\textit{eae}) and enterohaemolysin (\textit{hlyA}) and also O group-specific genes (Paton & Paton, 1998; Perelle et al., 2004, 2005). The PCR mixes were prepared and the cycling and subsequent electrophoresis conditions were those described by Paton & Paton (1998). The isolates were assigned H types based on restriction fragment length polymorphism of the PCR products (Fields et al., 1997; Zhang et al., 2000).

**Statistical analysis.** Data were analysed with Generalized Linear Mixed Models (GLMMs), fitting epidemiologically relevant factors such as region and season as fixed effects and factors defining the clustered sampling design as random effects, using Genstat for Windows v8 (VSN International). Mean prevalences were back transformed to the original scale using a variant of the method of Condon et al. (2004) to allow for the influence of random effects on the mean prevalence. Where the prevalence was small and positive samples were highly clustered, a random effects model was fitted to the left censored data and the results were weighted to reflect the likely proportion of negative lots in the sample. Where GLMMs failed to converge due to uniform effects, the StatXact v6 (Cytel Software) algorithm for Fisher’s exact test was applied to appropriately defined contingency tables, aggregated to whatever sampling stratum was most appropriate given the properties of the data. Excel 2003 was
used for general data management, both of the raw data and of the results.

RESULTS AND DISCUSSION

Test detection sensitivity

Faeces from the first 100 recta was tested as both 1 g and 10 g samples and differences in recovery of the target E. coli strains were examined using a two-tailed binomial test. There was no statistical evidence of a difference in recovery by weight of faecal sample (P=0.35) though, for this subset of rectal samples, more target group E. coli isolates were isolated from 1 g faecal samples than from 10 g samples. The testing of a second 1 g sample increased the recovery of target serogroups so that improvement in test detection based on IMS was at least as likely to be achieved by increasing the numbers of 1 g samples tested per faecal sample rather than by testing larger single amounts such as 10 g. The testing protocol in the study therefore continued with duplicate testing of 1 g faecal samples at animal level where sufficient faeces was available. O26 test results from TBX and rhamnose plates were consolidated for statistical analysis.

Recovery rates for the target E. coli serogroups

From the 1082 recta, there were 37 animals (3.4 %) with at least one sample positive for non-sorbitol-fermenting E. coli O157. Statistically significant differences were found for different age classes of animal at different times of year; it was therefore necessary to weight the mean prevalences seen in different classes by their incidence in the overall population of animals at slaughter, using population data for years 2005 and 2006 from Quality Meat Scotland. The prevalence of E. coli O157 carriage by slaughter sheep in Scotland was 3.4 % with a 95 % confidence interval (CI) of 0.7–9.6 %. This result is consistent with a crude 2.2 % prevalence in sheep faeces reported by Chapman et al. (1997) and the 1.7 % described by Paiba et al. (2002).

Fifty-six animals (5.2 %) were positive for E. coli serogroup O26, with statistically significantly higher prevalences being seen in samples collected between July and September (P=0.003). The between-lot variability in these samples was notably high. The estimated mean prevalence for the autumn, winter and spring seasons was 2.4 % with a 95 % CI of 0.03–8.7 %, while that for the summer season was 11.4 % with a 95 % CI of 0.3–35 %. The CIs are wide because of the extremely large between-lot variance component. Twenty-five animals (2.3 %) were positive for E. coli serogroup O103, giving an estimated prevalence of 2.3 % with a 95 % CI of 1.6–3.4 %, and one animal (0.1 %) was positive for E. coli serogroup O145, giving an estimated mean excretion prevalence of 0.1 % and a 95 % CI of 0.002–0.5 % (Fig. 1). Serogroup O111 strains were not isolated during the work, giving an estimated prevalence of 0 % with a 95 % CI of 0–0.3 %, and no sorbitol-fermenting E. coli O157 was recovered in the last month. The distribution of positive animals for each serogroup by abattoir is shown in Fig. 2.

Virulence profiles of the E. coli strains

E. coli O157. The virulence profiles of the E. coli O157 isolates recovered from within single samples were identical and so 37 virulence profiles were described from 37
Animals (Fig. 3). The majority of \( E. coli \) O157 isolates were verocytotoxin-positive with one animal (3%; 95% CI 0.1–14%) carrying only \( E. coli \) O157 \( vtx1 \)-positive isolates, 28 animals (76%; 95% CI 59–88%) carrying only \( vtx2 \)-positive isolates and two (5%; 95% CI 0.7–18%) animals possessing isolates that were both \( vtx1 \)- and \( vtx2 \)-positive. However, six animals carried verocytotoxin-negative \( E. coli \) O157. There was no evidence that the observation of \( vtx1 \) and \( vtx2 \) in an isolate was anything other than statistically independent (\( P = 0.28 \)). Similar isolates were common in a study of Scottish sheep (Solecki et al., 2009) and thus the carriage of verocytotoxin genes appears less frequent in \( E. coli \) O157 sheep isolates than in those from humans and cattle where approximately 99% of isolates are verocytotoxin-positive (Chapman et al., 1997; Locking et al., 2006; Gunn et al., 2007). All the \( E. coli \) O157 isolates were of flagellar type 7, confirming the strains to be serotype O157: H7, and all were positive for \( eae \). Thirty-one animals (84%; 95% CI 68–94%) carried isolates that were positive for \( hlyA \). E. coli O26, O103 and O145 isolates. There was limited carriage of verocytotoxin genes amongst the non-O157 \( E. coli \) isolates (Fig. 3). This is consistent with the findings of Aktan et al. (2004), who reported only five verocytotoxin-positive strains amongst 1227 archived \( E. coli \) isolates collected from animals at slaughter in the UK.

Verocytotoxin gene carriage was most frequent in the \( E. coli \) isolates of serogroup O26 that were recovered from 56 individual samples. Those recovered from the same animals were of the same \( vtx1 \), \( vtx2 \), \( eae \) and \( hlyA \) gene profiles except from one lamb carrying both \( vtx1 \)-positive and \( vtx2 \)-negative \( E. coli \) O26 strains. At animal level there were five animals (9% of O26 positive samples; 95% CI 3–20%) carrying only \( vtx1 \)-positive isolates; one (2%; 95% CI 0.05–10%) animal carried only \( vtx2 \)-positive isolates; and four animals (7%; 95% CI 2–17%) carried isolates that were both \( vtx1 \)- and \( vtx2 \)-positive. Fifty-five isolates were shown by PCR testing to be serotype O26: H11 and the remaining two were untypable by the method used here. All the 57 \( E. coli \) O26 isolates were positive for \( eae \) and 36 (63%; 95% CI 49–76%) were also positive for \( hlyA \). It is notable that there were marked differences in the virulence profiles of the \( E. coli \) O26 serogroup isolates dependent on the culture medium used, and CT-RMac was the more effective medium for isolating verocytotoxin-positive serogroup O26 strains, as previously reported (Evans et al., 2008).

Isolates of \( E. coli \) serogroup O103 were recovered from 25 samples and all were verocytotoxin-negative, with a 95% CI of 0–14%; seven (28%; 95% CI 12–49%) of these isolates were positive for both \( eae \) and \( hlyA \) (Fig. 3). This equates to a highly statistically significant (\( P < 0.001 \)) positive relationship between carriage of \( eae \) and \( hlyA \) genes in serogroup O103 isolates and the lack of verocytotoxin genes is consistent with strains identified in orphan lambs in England (Aktan et al., 2007). Eight isolates were shown to be \( E. coli \) O103: H2 and one was \( E. coli \) O103: H32. The single \( E. coli \) O145 isolate was negative for verocytotoxin genes, but positive for \( eae \) and \( hlyA \).

Seasonality and animal age influence on \( E. coli \) O157: H7 isolations

\( E. coli \) O157: H7 was not isolated from rectal samples collected from January to March 2006 and the mean faecal shedding prevalence in this period was thus significantly lower than in other quarters of the year (\( P < 0.001 \)). This seasonality was consistent with previous work in a Sheffield abattoir, where 17 out of the 22 isolations of \( E. coli \) O157

\[ \text{Fig. 2. Number of } E. coli \text{ O157, O26, O103 or O145 isolated from animals by abattoir.} \]
from sheep were made during the months of June–September inclusive (Chapman et al., 1997), and with evidence from a slaughterhouse study that revealed higher faecal shedding rates by sheep in the period of June, July and August (Paiba et al., 2002). Excepting winter, there were statistically significant differences in the mean shedding rates associated with lambs and with adults or hoggs \( (P=0.003) \). The mean O157 shedding prevalence of lambs presented for slaughter in Scotland during April–December was 3 % with a 95 % CI of 0.005–13 %. The mean O157 shedding prevalence of adults or hoggs presented for slaughter during this period was 10 % with a 95 % CI of 4–17 %.

**Quantitative studies**

There were 28 animals that were carrying verocytotoxin-positive *E. coli* O157: H7 where there was sufficient faeces to carry out direct plating and bacterial enumeration. In samples from 16 (52 %) of these positive animals, *E. coli* O157: H7 was not detected by direct plating, suggesting low level carriage of these target organisms detectable only by IMS. In seven animals, the estimated mean bacterial counts were at \( 1 \times 10^3 \) c.f.u. g\(^{-1}\) or above and one of these samples yielded a count of \( 1.15 \times 10^7 \) c.f.u. g\(^{-1}\) of *E. coli* O157: H7. This demonstration of high level shedding was consistent with results from cattle (Chase-Topping et al., 2008); such animals are considered important for maintaining and spreading the organism within herds (Matthews et al., 2006). There are few other reports of the concentration of this bacterium in sheep faeces, but Ogden et al. (2005) found 20 of 44 *E. coli* O157-positive sheep shedding at \( 1 \times 10^3 \) c.f.u. g\(^{-1}\) or above.

There were eight animals positive for *E. coli* of serogroup O26: H11 that were verocytotoxin-positive with sufficient faeces to enumerate the target organism. In samples from seven (88 %) positive animals, the organism was not detected by direct plating, again suggesting low level carriage. In the remaining animal, the estimated mean bacterial count was \( 7.54 \times 10^4 \) c.f.u. g\(^{-1}\).

**Distribution of positive animals**

There was no evidence of any variation in the faecal carriage of the specific *E. coli* serogroups across the four abattoirs. Irrespective of abattoir, the positive isolations of *E. coli* O157: H7 were highly clustered, with the most extreme clustering arising in nine positive samples collected on a single date in May 2006 from the abattoir in Greater Glasgow. Our finding of a clustering of *E. coli* O157: H7 is consistent with the findings of Ogden et al. (2005), who reported the isolation of *E. coli* O157 from 44 of 676 animals where 30 of the positive isolations came from a sampling of 33 sheep in one flock. This clustering of positive results is consistent with the assumption in the study design of multiple classes of flock with very different within-flock prevalences.
Similarity of isolates to those causing human infections

There were 31 samples where isolates of \( E. coli O157 : H7 \) were \( vtx-, eae- \) and \( hlyA \)-positive and hence the majority of \( E. coli O157 \) isolates were consistent with those causing human disease (Kaper et al., 2004). Weighting by slaughter volume, the highest risk for carriage of \( E. coli O157 : H7 \) by slaughter sheep in Scotland was during the months of July–September and by sheep that were adults or hoggs (lambs become hoggs on 1 January the year following birth). Internationally, Scotland has a high rate of \( E. coli O157 : H7 \) in humans with infections peaking during summer months (FSA, 2000). Since the late 1980s, the rates of human infection by \( E. coli O157 : H7 \) have been substantially and constantly higher in certain regions of Scotland than in England and Wales (FSA, 2000). The work therefore sought to identify any regional variation in carriage rates of \( E. coli O157 \) by sheep to complement our knowledge of potential risks to human health. The mean number of isolations was lowest from the Grampian abattoir, which is sited in the region of Scotland with consistently highest rates of human infection. The work found no statistical evidence of any variation in the mean levels of faecal carriage of \( E. coli O157 : H7 \) by abattoir (\( P=0.42 \)) and animal age and season had a greater impact on the observed distribution than the regional sampling. These findings do not suggest that cases of \( E. coli O157 : H7 \) are higher in any particular Scottish region as a direct consequence of a higher rate of faecal carriage in sheep in that region.

In Continental Europe, the most common non-O157 verocytotoxigenic \( E. coli \) serogroups causing human disease are \( E. coli O111, O26, O103 \) and \( O145 \) (WHO, 1998), which have been reported in 11, 11, 7 and 5 countries, respectively (Eklund et al., 2001; Caprioli et al., 1997). However, this study found no verocytotoxin-positive strains amongst the \( E. coli \) serogroup \( O103 \) or \( O145 \) isolates recovered from sheep in Scotland. This is similar to our findings with Scottish beef cattle (Pearce et al., 2006). It is also consistent with the findings of Aktan et al. (2004), who reported no verocytotoxin-positive \( E. coli \) strains from amongst 401 random isolates recovered from sheep at slaughter in England and Wales. The absence of \( E. coli \) \( O111 \) shedding was consistent with the results of our cattle prevalence study (Pearce et al., 2006) and is in agreement with our previous work in Scotland that included testing by DNA hybridization. We did not detect sorbitol-fermenting \( E. coli O157 \) (SFO157) from the 83 recta received during the last month of the study when the study protocol had been altered to investigate SFO157 carriage in sheep in response to this organism being isolated from children in Scotland between April and May 2006 (Pollock et al., 2010).

Nevertheless, the results show that sheep presented for slaughter in Scotland may carry non-O157 strains of \( E. coli \) that can be presumed to have potential to cause human infection. The strains that are \( eae- \)positive but \( vtx- \)negative may be less likely than verocytotoxin-positive strains to cause human disease (Ramachandran et al., 2003). Verocytotoxin-positive isolates were particularly identified in \( E. coli O26 : H11 \) where 11 samples carried strains possessing \( vtx, eae \) and \( hlyA \) virulence genes. Though infrequent, occurring in five samples only, the \( E. coli \) serogroup \( O26 \) strains that are \( vtx- \)positive and \( eae- \)positive are consistent with those causing severe human disease (Boerlin et al., 1999). Having established the carriage of such \( E. coli O26 \) strains in sheep in Scotland, with the consequent risk of virulent infections occurring in humans, enhanced surveillance of human infections should be maintained so as to monitor future occurrence and the threat to human health from these strains.

In conclusion, our results show that sheep slaughtered in Scotland carry a range of \( E. coli \) serogroups carrying virulence genes that are highly associated with a spectrum of diarrhoeal disease in humans irrespective of time of year of slaughter. \( E. coli \) from sheep may present a risk for human infections and should continue to be considered as a vehicle of infection in outbreak investigations.

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