REPORT TO FSA SCOTLAND

ON

PROJECT S14005.

PREVALENCE AND CONCENTRATION OF *ESCHERICHIA COLI* SEROTYPE O157:H7 AND OTHER VTEC IN SHEEP PRESENTED FOR SLAUGHTER IN SCOTLAND.

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1. LAYPERSONS SUMMARY

For the FSA research requirement S14R0003, the Scottish Agricultural College (SAC) led a group of researchers to test sheep at slaughter in Scotland for the bacterium *Escherichia coli* O157 and similar bacteria that produce poisons called verocytotoxins. Together, these organisms are called verocytotoxigenic *E. coli* (VTEC). This research was carried out to improve our knowledge of how often *E. coli* O157 or other VTEC occur in the dung of sheep at slaughter. This is important as *E. coli* O157 causes human disease and there are some areas of Scotland where the number of human cases is much higher than in others. The study was performed to find out how often *E. coli* O157 and the major non-O157 VTEC, that are mostly likely to cause serious human infection, occurred in sheep at slaughter, and if there were seasonal changes, or differences by region.

The project was successful in sampling 1,082 sheep at slaughter in Scotland at four different slaughterhouses from July 2005 to June 2006. The prevalence determined for *E. coli* O157:H7 carriage by slaughter sheep in Scotland was 3.4% with a 95% confidence interval (0.7%, 9.6%). This detection rate is similar to the crude level of 2.2 positive for every 100 sheep tested that was previously reported by Sheffield based researchers (Chapman *et al.* 1997) and the 1.7 positive out of 100 sheep tested, reported by the Veterinary Laboratories Agency (Paiba *et al.* 2002). Importantly, our results showed *E. coli* O157 was more commonly isolated from adult and year old sheep than from lambs. Also, there were no isolations in January, February or March 2006. The highest risk for carriage of *E. coli* O157 by slaughter sheep in Scotland was during the months of July to September and by sheep that were adults or one year old. The work found no statistical evidence of any variation in the numbers positive at the four slaughterhouses. Crucially, our findings do not support the idea that human cases of *E. coli* O157 are higher in any Scottish region as a direct consequence of a higher rate of carriage in sheep in that region. There were 33 animals positive for *E. coli* O157:H7, and in seven animals the actual number of *E. coli* O157 was more than 1,000 per gram of faeces. This is similar to results from cattle where animals, termed supershedders, produce these high numbers of *E. coli* O157 in the dung, and these are important for the spread of the bacterium in groups of animals.

The work on the similar organisms, referred to as non-O157 VTEC types, focussed on those causing human disease in Europe, often of the types called O111, O26, O103 and O145. In this study the organisms of groups O103, O111 or O145 were mostly incapable of producing the verocytotoxin poison. The type called *E. coli* O26 was found with 11 of 64 isolates positive for verocytotoxin, but in Scotland comparing the results to those from a previous study of cattle suggests that sheep are less likely than cattle to be a source of the VTEC O26 strains.

2. SUMMARY

The project successfully sampled sheep at slaughter in Scotland and carried out testing for *Escherichia coli* O157:H7 and other non-O157 verocytotoxigenic *E. coli* (VTEC). This research has increased our knowledge of the presence of *E. coli* O157:H7 in sheep faeces in Scotland and provided information on the presence of non-O157 VTEC strains.

Importantly, the findings are of highly clustered isolations of *E. coli* O157:H7. Significant differences were identified in *E. coli* O157:H7 carriage by animal age group; where strains were more commonly isolated from adult and hogg classes of animals than from lambs. Additionally, *E. coli* O157:H7 was not isolated during the months of January, February or March 2006. The mean *E. coli* O157:H7 excretion frequency for lambs presented for slaughter in Scotland during April to December was 3% with a 95% confidence interval (0.005%, 12.6%). The mean frequency of *E. coli* O157:H7 excretion by adults or hoggs presented for slaughter in Scotland during April to December was 10% with a 95% confidence interval (4%, 17%). The crude detection rate of *E. coli* O157:H7 calculated from the weighted contribution of these means with respect to the relative proportions of adults, hoggs and lambs slaughtered at different times of year allows an estimate of the mean frequency of *E. coli* O157:H7 excretion. The prevalence of *E. coli* O157:H7 carriage by slaughter sheep in Scotland is 3.4% with a 95% confidence interval (0.7%, 9.6%). This result is consistent with the crude 2.2% prevalence in sheep faeces reported by Chapman *et al.* (1997) and the 1.7% described by Paiba *et al.* (2002). The highest risk for carriage of *E. coli* O157:H7 by slaughter sheep in Scotland was during the months of July to September and by sheep that were adults or hoggs.

The work found no statistical evidence of any variation in the faecal carriage of *E. coli* O157:H7 by abattoir ($p=0.42$) and age and season have a greater impact on prevalence than the regional origin of the flock. Crucially, our findings do not support the hypothesis that human cases of *E. coli* O157 are higher in any particular Scottish region as a direct consequence of a higher rate of carriage in sheep in that region.

There were 33 animals positive for *E. coli* O157:H7 and in seven animals the bacterial counts were at 1×10^3 CFU/g or above and with one sample yielding a count of 1.15×10^7 CFU/g of *E. coli* O157:H7. This demonstration of sheep with bacterial counts in faeces at 1×10^3 CFU/g or above is consistent with results from cattle where animals exist, termed supershedders, which shed high levels of *E. coli* O157:H7 and are considered an important pre-determinant for the clustering of positives within groups.

In Continental Europe, the most common non-O157 VTEC 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 and Tozzi, 1998). In this study we found very limited carriage in sheep in Scotland of verocytotoxin positive strains amongst the potential VTEC serogroups of O103, O111 or O145.

The estimated mean excretion rate for *E. coli* O26 by plating to TBX media was 3.4% with a 95% confidence interval of (2%, 5%). The estimated mean excretion frequency of *E. coli* O26, by plating to rhamnose selective media, was 4% with a 95% confidence interval of (3%, 6%). Thus, in Scotland, sheep appear less likely than cattle to be a source of VTEC O26 strains. It remains unclear why the numbers of *E. coli* O26 infections in humans in Scotland are so much lower than infections caused by *E. coli* O157:H7 (Locking *et. al.,* 2006) when it is apparent that there is carriage of *E. coli* O26 strains in cattle and sheep in Scotland and these strains are potentially virulent for humans.

In conclusion, we have provided a benchmark for shedding of *E. coli* O157:H7 by sheep in Scotland. Sheep appear less likely than cattle to be a source of verocytotoxigenic *E. coli* of serogroup O26 strains and we have produced evidence that VTEC strains of serogroups O103, O111 or O145 are of low prevalence or absent from sheep at slaughter. Despite the carriage of *E. coli* O26 strains in sheep in Scotland that are potentially virulent for humans the results of enhanced surveillance continues to show *E. coli* O157:H7 strains as the greatest threat to human health. The highest risk for carriage of *E. coli* O157:H7 by slaughter sheep in Scotland was during the summer months of July to September and by sheep that were adults or hoggs. Crucially, our findings do not support the 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. Evidence was produced that supershedders for *E. coli* O157:H7 exist in sheep populations, and these animals are presumed to be the reason for the marked clustering of positive samples.

3. STUDY BACKGROUND

The study background was part of the research application to FSAS and is reproduced here to provide a scientific context for the report. Verocytotoxin (vtx) producing *Escherichia coli* (VTEC), and particularly strains of serotype O157:H7, have emerged as significant food poisoning pathogens of humans where the possibly fatal outcomes of infection are haemorrhagic colitis (HC), and haemolytic uraemic syndrome (HUS) (Ammon 1997). All VTEC strains cause disease through production of potent phage-encoded cytotoxins termed verocytotoxins (producing either

vtx$_1$ or *vtx*$_2$ or both). Another virulence-associated factor that may be expressed by VTEC is intimin, a surface protein, responsible for the intimate attachment of VTEC to intestinal epithelial cells resulting in attaching and effacing (AE) lesions (Roe and Gally, 2000). Intimin is encoded by the chromosomal gene *eae*.

Internationally, Scotland has a relatively high rate of *E. coli* O157:H7 related disease in humans with the peak of cases of infection occurring during summer months. Since the late 1980s, rates of infection in certain regions of Scotland have been substantially and constantly higher than in England & Wales (FSA, 2000). Since domestic ruminants, especially cattle and sheep, are major reservoirs of VTEC and human infections may arise through a variety of exposures (Locking *et al.*, 2000) there have been many studies to examine *E. coli* O157:H7 carriage by cattle. Definitive work by SAC has shown at least one shedding animal in 23% of Scottish cattle herds (Synge and Paiba, 2000) but with no detectable regional variation.

In clarifying the risks for human infections arising from animal reservoirs, most epidemiological work in Scotland has focused on cattle. This is even though sheep have been proposed as sources for human infection on a number of occasions. Contact with lambing ewes was linked to human illness (Allison *et al.,* 1997) and sheep faeces apparently contaminated the water supply at a campsite outbreak (Licence *et al.,* 2001) and caused environmental contamination and an outbreak at a boy-scout camp (Strachan *et al.,* 2001). However, as a consequence of a lack of epidemiological work, there is less knowledge of *E. coli* O157:H7 carriage in sheep compared to cattle. Reports suggest that at abattoir the prevalence of *E. coli* O157:H7 in sheep faeces is lower than in cattle faeces (Chapman *et al.,* 1997; Paiba *et al.,* 2002) but there is little information available on regional or seasonal variation. A recent SAC study examined fifty sheep flocks randomly selected from all regions of Great Britain (Synge – personal communication). One or more *E. coli* O157 positive samples were identified from four (8%) of the flocks with 95% confidence limits of 2.2% to 19.2%. This suggests the within flock prevalence of *E. coli* O157:H7 in sheep is variable and as a result the study was too small to identify any regional variation. Consistent with this is the limited evidence of regional variation previously found at abattoir (Paiba *et al*., 2002). Regarding seasonal variation, in a Sheffield abattoir study, 17 out of the 22 *E. coli* O157 isolations from sheep were made during the summer (Chapman *et al.,* 1997) and there was also evidence of higher faecal shedding by sheep in the summer in a slaughterhouse study (Paiba *et al.,* 2002). A personal communication from Iain Ogden, University of Aberdeen, suggested high numbers of shedding sheep were found in Grampian in the summer of 2003. Recent cattle studies, (Omisakin *et al.,* 2003; Ogden *et al*., 2004) have shown a trend towards higher numbers of animals shedding in the winter, though analysis is confounded by the

considerable variability within herds, and at herd level no differences were found between summer and winter (Ogden *et al*., 2004).

From the above details it is clear that there is a need to improve epidemiological information on carriage of *E. coli* O157:H7 by sheep that may be linked to the high regional variation seen in human cases in Scotland. However, it is apparent from studies of cattle conducted by SAC that the prevalence of *E. coli* O157:H7 within herds is strikingly variable. In SAC work, 78% of 952 farms had no animals with *E. coli* O157:H7 shedding but a small proportion, approximately 2% of farms, had very high prevalences of infection (with 90-100% of samples being positive) (Matthews *et al.,* 2006a). In such farms, the presence of cattle, called "supershedders", that shed high levels of *E. coli* O157:H7 are an important pre-determinant for the clustering of positives within groups (Matthews *et al.,* 2006b; Low *et al.,* 2005). These "supershedders" are considered to be major drivers in the persistence of the organism in herds.

In any proposal the consideration of within flock prevalence is a vital factor in prevalence estimates and must be included in studies of regional and seasonal variation. It is likely that the variability already identified in flock prevalence is linked to the occurrence of "supershedders". Previous work has shown that in one examined flock the majority of selected faecal samples were positive (Strachan *et al.,* 2001) and in the 50 flocks examined by SAC, one positive sample was identified in three flocks, and eight positive samples in a fourth. In the single flock study a minority of ewes shed at levels between 10^3 and 10^5 CFU/g (Strachan *et al.,* 2001) and in the SAC work enumeration showed most positive samples were $<10^3$ CFU/g. It was intended that this proposal should develop epidemiological information on sheep faecal carriage at slaughter and enumerate *E. coli* O157:H7 so as to increase our knowledge of shedding levels and to allow an examination for regional and seasonal variation that complements our knowledge and identifies potential risks to human health.

VTEC strains causing human infections belong to a large number of O and H serotypes in addition to O157:H7. Despite problems of detection, many non-O157 VTEC serotypes have been associated with illness in humans (Blanco, *et al.,* 2001). *E. coli* serogroup O26 is considered the second most common cause of HUS in UK (Smith *et al.,* 2001) and serogroups O26, O103, O111 and O145 are regarded as especially likely to cause severe human infections (Boerlin, *et al.,* 1999; Schmidt, *et al.,* 2001). Severe disease, including HC and HUS, has been statistically associated with strains carrying the eae gene for intimin and producing vtx_2 (Boerlin, *et al.,* 1999) and these are particularly found in serotypes: O26:H11, O103:H2 and O111:NM (WHO, 1998). The Study of Infectious Intestinal Disease in England showed serotypes of non-O157 VTEC that are negative for the *eae* gene have been rarely implicated in severe human

illness and were more frequently found among asymptomatic carriers, in uncomplicated cases of diarrhoea, or among adult patients (FSA, 2000). Surveillance of childhood HUS in Scotland between 1997 and 2001 identified that 89% of cases had *E. coli* O157:H7 infection (Locking – personal communication). However, subsequent to a recommendation of the *E. coli* O157 Task Force Report the Scottish *E. coli* O157 Reference Laboratory (SERL) is receiving an increasing number of faecal samples to screen for VTEC. Few non-O157 strains causing HUS have been identified but the real contribution of non-O157 isolates to diarrhoeal disease is unclear. In 2003, SERL received or recovered 31 non-O157 *E. coli* isolates from 30 individuals. Of these isolates 16 (51.6%) possessed virulence genes (*vtx*$_1$, *vtx*$_2$, *eae* or the haemolysin gene - *hlyA*).

Recently SAC conducted a national survey for FSA (Scotland) to determine the prevalence of *E. coli* O26, O103, O111 and O145 shedding in cattle. Scottish store and finishing cattle were sampled on 338 Scottish farms and the proportion of farms on which shedding was detected was 15.3% for O157, 19.8% for *E. coli* O26, 20.3% for *E. coli* O103, and 7.1% for *E. coli* O145. No *E. coli* O111 was detected (Pearce *et al*., 2006). Shedding of *E. coli* O26, O103 and O145 was more common in summer and autumn but there was little evidence for any regional variation. Carriage of the virulence gene *vtx* was rare in *E. coli* O103 and O145 isolates but 49.0% of *E. coli* O26 isolates were *vtx* gene positive. The *eae* gene was present in *E. coli* isolates belonging to serogroup O26 (83.9%), serogroup O103 (37.9%) and serogroup O145 (83.7%). The *hlyA* gene was also common, being found in serogroup O26 (51.8%), serogroup O103 (36.7%) and serogroup O145 (65.1%) isolates. The genes *eae* and *hlyA* were highly associated in all three serogroups. Among *E. coli* O26 isolates, 28.9% had *vtx*, *eae* and *hlyA* — a profile consistent with *E. coli* O26 strains known to cause human disease and isolates from both cattle and humans were shown to be closely related (Lesley Allison - personal communication).

There are also a number of reports that non-O157 VTEC are common in sheep faeces often exceeding 40% of animals sampled and with multiple serotypes present in individual animals (Beutin *et al.,* 1993; Kudva *et al.,* 1997; McCluskey, *et al.,* 1999; Djordjevic, *et al.,* 2004). A recent study (Djordjevic, *et al.,* 2004) identified 248 VTEC isolates, comprising 49 serotypes from 196 sheep. The serotypes O5:H-, O75:H8, O91:H-, O123:H- and O128:H2 have been considered to be common ovine serotypes though only 5% of isolates carried *vtx* and *eae* genes. In contrast, in the UK there is little detail available on common serotypes. In recent years, the only work has been to examine 1,227 *E. coli* isolates for carriage of *eae* and this identified that 18% of *E. coli* recovered from sheep were *eae* positive though very few of these were *vtx* positive. In contrast to other reports common serogroups in UK have been suggested as: O26, O56, O103 and O156 (Martin Woodward, Defra VLA - personal communication).

Despite this knowledge there is no information on shedding of non-O157 VTEC by sheep in Scotland. This study therefore proposed to determine the prevalence in slaughter sheep of the major VTEC serogroups isolated from human cases that are mostly likely to cause serious infection, and have recently been examined in bovine sources in Scotland.

4. STUDY OBJECTIVES

The primary research objectives in order of priority agreed with FSAS were:

Objective 1. To validate the sensitivity of the IMS method for 1g sample sizes.

Objective 2. To establish the frequency of excretion of *E. coli* O157:H7 in faeces of sheep presented for slaughter in Scotland.

Objective 3. To enumerate the *E. coli* O157:H7 excreted in faeces of sheep presented for slaughter in Scotland.

Objective 4. To examine for the presence of non-O157 VTEC serotypes likely to cause human disease in faeces of sheep presented for slaughter in Scotland.

Objective 5. To establish if there is regional or seasonal variation in the frequency and level of excretion of *E. coli* O157:H7 in faeces of sheep presented for slaughter in Scotland.

5. STUDY DESIGN

The study design for FSAS was developed in light of practical constraints, current knowledge and expert opinion of the likely prevalence of faecal shedding of *E. coli* O157:H7 in sheep. The design included an important assumption of a trimodal distribution of flock prevalences. Thus, calculations were based on an overall national prevalence of 2% of sheep being *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 and where 75% of animals may be positive. The remaining 92% of flocks are negative. The estimated mean prevalence under various sampling assumptions has been calculated. Simulating data from this model, the confidence intervals around the predicted mean are estimated using a non-parametric bootstrap approach, to fully allow for the sampling variability between the three populations. As would be expected, the confidence intervals become narrower as the sampling effort, both between and within lots, increases.

Sampling a single animal per abattoir lot or flock would not distinguish between the low and negative prevalence groups. However, to do this adequately requires a very high sampling rate (30 samples per lot) which was impracticable. To distinguish between the high and negative prevalence lots, at least two samples should be collected from each lot but since there is enormous scope for variability between lots, it was recommended that the study collects 5 random samples from each lot. Power calculations indicated that collecting 5 samples from 250 lots could identify a prevalence of 2.4% (95%CI: 0.8% - 3.7%).

We considered it impracticable to sample all 22 abattoirs in Scotland and believed that examining a subset most likely to have local supplies of sheep would assist in identifying if any regional differences in prevalence exist. Since there are marked regional differences in human infection rates we proposed to select 4 abattoirs from different regions throughout Scotland. There is no requirement for the sample numbers to be pro-rata to the numbers of sheep slaughtered as the objective was to determine the prevalence in the slaughtered population of sheep and not for the population of animals entering the human food chain. (The latter objective would require samples to be weighted pro rata to the slaughter rate).

The testing procedure for *E. coli* O157 detection used bacterial enrichment of 1 g faeces samples in buffered peptone water (BPW) with subsequent immunomagnetic separation (IMS) as in our previous epidemiological work (Pearce, *et al.,* 2004a & b; Pearce *et al*., 2006). In addition, 10% of samples were tested in duplicate and to fulfill Objective 1 the first 100 samples were also tested as 10 g amounts.

Methods in the literature for the detection of non-O157 *E. coli* strains have varied considerably, being either examination of individual bacteria for verocytotoxic effect, or carriage of genes encoding *vtx*, or alternatively the direct recovery of VTEC from faecal samples by *vtx* gene detection using replica colony plating and DNA hybridisation. The former methods are not applicable to large scale prevalence work and the latter technique has been used to examine faecal samples for the presence of non-O157 VTEC by the IPRAVE studies based at SAC Inverness and with Health Protection Agency, Colindale. However, the DNA hybridisation method was relatively insensitive, as 20 *E. coli* O157:H7 strains were isolated by IMS, whereas DNA hybridisation identified only 1 *E. coli* O157:H7 strain and no *E. coli* O103, O111, O145 strains from the same samples (Jenkins *et al.,* 2003; Pearce *et al.,* 2004b).

Though there have been reports that non-O157 VTEC are common in sheep faeces it was suggested that rather than attempting the detection of ALL non-O157 VTEC the work should target those serogroups most commonly associated with serious human infection (i.e. O26,

O103, O111 and O145). The advantages of this approach were that IMS beads are available for the five major serogroups (O157, O26, O103, O111 and O145) and the IMS technique has considerable advantage over the molecular methods, by offering greater sensitivity and specificity. The method is appropriate for large-scale prevalence studies of non-O157 VTEC and results can be related to those of the FSA (Scotland) funded work on prevalence of non-O157 serogroups in cattle (FSA S01014). In addition, the isolates were characterised for flagellar type and for possession of those virulence determinants (*vtx, eae, hlyA*) that are commonly associated with serious human infections (Boerlin *et al.,* 1999).

The above study design would adequately meet the objectives 1, 2, 3 and 4. However, for objective 5 it is apparent that when considering seasonal and regional effects, the main determinant of changes in prevalence will be the proportion of flocks/lots sampled that include large numbers of positive animals. Assuming this value fluctuates around the assumed prevalence of 2% the study would require 2,352 lots of sheep and 12,000 samples to be examined to distinguish between regional or seasonal differences that are between 2% to 5%. Thus, the study required an extremely large number of sampling occasions to identify any but the largest statistically significant differences between different seasons and regions, and consumables costs alone for this amount of work would have made the project unaffordable. It was therefore recommended that the survey be explicitly powered on the basis of the desired properties of the prevalence estimate i.e. objectives 2, 3, and 4. Samples would be collected in a way that ensured region and season were not confounded, and that the data was analysed to explore whether any possible trends would merit further study so meeting the objective 5 requirements.

6. MATERIAL & METHODS

6.1. Abattoir and animal selection

Quality Meat Scotland (QMS) identified four abattoirs in four separate regions of Scotland to participate in the study. The chosen regions that consistently report high rates of human infection by *E. coli* O157:H7 and in which abattoirs were visited were Grampian and Dumfries and Galloway. Abattoirs in Greater Glasgow and Highland were also visited, where human infection rates tend to be about the same or lower than the national average. The visits were made on a rotational basis at weekly intervals from July 2005 to June 2006. Five separate and random lots of sheep for slaughter were chosen from amongst the lots submitted to the abattoir on the visit day. After killing, the recta were collected on the slaughter line from up to five randomly chosen individuals from each lot. The recta were individually tied, bagged, labelled and

then placed in a second bag in chill boxes. Where available, animal ear tag information was also collected for each chosen individual animal. The numbers of animals according to age and season of sampling are shown for each abattoir in Table 1.

Ages of animals were categorised as: adult, hogg or lamb. It must be noted that a hogg is legally defined as unshorn sheep after 31st December that were born in that immediately preceding year. It is notable from examining Table 1 that no animals were recorded as hoggs or adults at the Highland or Grampian abattoirs. The absence of adults is because these abattoirs almost exclusively provide prime lamb through contracts to major retailers and these contracts would exclude any adult animals. The absence of sheep from the hogg classification is almost certainly because of colloquialisms used in the sheep trade and at these abattoirs the lamb category includes “late lambs” that elsewhere would be referred to as hoggs.

6.2. Samples

Laboratory work included the examination of multiple samples from the majority of recta. The recta taken from slaughter sheep were couriered to Inverness in chill boxes and tested 24 hours after collection (± 20 hours). Two hundred and fifty seven recta were collected from the abattoir based in Greater Glasgow, 261 from the Dumfries and Galloway abattoir, 270 from the Highland based abattoir and 294 recta from the abattoir sited in Grampian. The 1,082 recta were opened individually and faeces collected aseptically for culture. Where there were insufficient faeces an aggressive swab was taken from the rectum wall termed "gut swab". The total number of tests was 2,086 that comprised; 1,182 samples of 1 gram (g) faeces or gut swab samples; 751 duplicate 1g samples, 100 replicate 10g samples. For 53 recta, a swab was also taken from the rectal anal junction and termed "rectal swab" (Table 2).

Table 1. Ages of animals sampled at each abattoir visit

Submitting abattoir: Greater Glasgow					
	Season				
Age	1st quarter	2nd quarter	3rd quarter	4th quarter	Grand Total
Adult	40	19	20	15	94
Hogg	20	0	0	40	60
Lamb	15	26	48	14	103
Unknown	0	0	0	0	0
Grand Total	75	45	68	69	257
Submitting abattoir: Dumfries & Galloway					
	Season				
Age	1st quarter	2nd quarter	3rd quarter	4th quarter	Grand Total
Adult	5	0	5	5	15
Hogg	16	3	2	44	65
Lamb	46	59	53	0	158
Unknown	4	0	0	19	23
Grand Total	71	62	60	68	261
Submitting abattoir: Highland					
	Season				
Age	1st quarter	2nd quarter	3rd quarter	4th quarter	Grand Total
Adult	0	0	0	0	0
Hogg	0	0	0	0	0
Lamb	55	65	75	75	270
unknown	0	0	0	0	0
Grand Total	55	65	75	75	270
Submitting abattoir: Grampian					
	Season				
Age	1st quarter	2nd quarter	3rd quarter	4th quarter	Grand Total
Adult	0	0	0	0	0
Hogg	0	0	0	0	0
Lamb	75	70	74	75	294
Unknown	0	0	0	0	0
Grand Total	75	70	74	75	294

Table 2. Number of recta collected from animals sampled at each abattoir visit and numbers of tests performed.

Sampling			Laboratory samples				
	Date	Animals	1g faeces	1g repeat	10g faeces	Gut Swab	Rectal Swab
Greater Glasgow	20 July 2005	15	12	2	0	15	0
	09 August 2005	11	10	1	2	11	11
	20 September 2005	19	14	13	0	5	0
	04 October 2005	25	19	15	0	6	0
	08 November 2005	20	16	14	0	4	0
	20 December 2005	23	14	12	0	9	0
	17 January 2006	19	14	13	0	5	0
	21 February 2006	25	17	10	0	8	0
	29 March 2006	25	19	12	0	6	0
	11 April 2006	25	19	16	0	6	0
	23 May 2006	25	22	17	0	3	0
	13 June 2006	25	18	16	0	7	0
		257	194	141	2	85	11
Dumfries & Galloway	19 July 2005	18	18	1	10	0	3
	16 August 2005	19	19	2	12	19	10
	27 September 2005	25	24	24	0	1	0
	10 October 2005	15	14	11	0	1	0
	15 November 2005	23	21	18	0	2	0
	01 December 2005	22	21	20	0	1	0
	10 January 2006	24	23	16	0	1	0
	28 February 2006	23	21	19	0	2	0
	21 March 2006	21	20	19	0	1	0
	25 April 2006	25	16	14	0	9	0
	16 May 2006	23	19	19	0	4	0
	27 June 2006	23	19	19	0	4	0
		261	235	182	22	45	13
Highland	01 August 2005	15	14	1	4	15	9
	30 August 2005	25	25	25	15	0	0
	13 September 2005	25	24	24	13	1	0
	25 October 2005	25	25	21	0	0	0
	01 November 2005	25	25	24	0	0	0
	06 December 2005	25	25	21	0	0	0
	24 January 2006	25	23	23	0	2	0
	07 February 2006	25	19	19	0	6	0
	14 March 2006	25	23	19	0	2	0
	18 April 2006	25	22	20	0	3	0
	09 May 2006	20	17	15	0	3	0
	27 June 2006	10	10	9	0	0	0
		270	252	221	32	32	9
Grampian	26 July 2005	20	20	2	12	20	17
	22 August 2005	25	25	3	16	25	3
	05 September 2005	25	25	24	16	0	0
	17 October 2005	25	25	25	0	0	0
	21 November 2005	24	22	20	0	2	0
	12 December 2005	25	25	20	0	0	0
	30 January 2006	25	19	19	0	6	0
	13 February 2006	25	21	19	0	4	0
	06 March 2006	25	22	19	0	3	0
	03 April 2006	25	23	22	0	2	0
	01 May 2006	25	21	17	0	4	0
	05 June 2006	25	21	17	0	4	0
		294	269	207	44	70	20
Totals		**1082**	**950**	**751**	**100**	**232**	**53**

6.3. Bacterial culture

The testing procedure for *E. coli* O157 detection used bacterial enrichment of 1 g faeces samples in buffered peptone water (BPW) with subsequent immunomagnetic separation (IMS). In addition, initially 10% of samples were tested in duplicate and the first 100 samples were tested as 10 g amounts. After IMS separation the magnetic beads were plated onto sorbitol MacConkey supplemented with cefixime and tellurite (CT-SMac). From CT-SMac plates up to 6 non-sorbitol fermenting colonies were plated to chromocult coliform agar and distinctive red-pink colonies tested by anti-*E.coli* O157 antibody coated latex reagents (Oxoid) for confirmation as *E. coli* O157.

The 1g faecal samples and swabs were suspended individually in 20ml buffered peptone water (BPW), whereas the 10g faecal samples were suspended in 225ml BPW. The BPW suspensions were incubated at 37°C (±1°C) for 6 hours and then separate 1ml aliquots were subjected to testing by immunomagnetic separation (IMS). To detect serogroups O26, O103, O111, O145 or serogroup O157 the serogroup specific paramagnetic beads (LAB M, Bury, Lancashire; Invitrogen, Paisley, UK respectively) were used. The IMS and non-O157 serogroup testing was carried out essentially as previously described (Jenkins *et al.,* 2003) with culture on chromocult TBX plates (Merk KgaA, Darmstadt, Germany). The *E. coli* O26 specific beads were also sub-cultured onto rhamnose MacConkey agar supplemented with cefixime (0.05mg/litre) and tellurite (2.5mg/litre) (CT-RMac, IDG, UK Ltd., Bury, Lancashire, UK). Suspensions of the serogroup O157 beads were sub-cultured onto MacConkey agar containing sorbitol, cefixime (0.05mg/litre) and tellurite (2.5mg/litre) (CT-SMac, Oxoid, Basingstoke, UK). All these media plates were incubated overnight at 37°C (± 1°C). From each chromocult TBX plate up to ten morphologically different colonies were tested against serogroup specific antisera (Statens Serum Institut, Copenhagen, Denmark) using a slide agglutination test. Colonies showing no fermentation of rhamnose growing on the CT-RMac agar were tested for agglutination against O26 antiserum. Sorbitol non-fermenting colonies on CT-SMac agar were sub-cultured onto chromocult coliform agar (Merk KgaA, Darmstadt, Germany) and incubated at 37°C (± 1°C) overnight. Pink colonies were tested for latex agglutination with anti-*E. coli* O157 latex beads (Oxoid, Basingstoke, UK). Presumptive positive isolates were subcultured onto MacConkey agar (Oxoid, Basingstoke, UK), then stored on Prolab beads (Prolab Diagnostics, South Wirral, Cheshire, UK) at -80°C (± 4°C).

All tested faeces samples were held at 4°C (± 2°C) and those from which positive O157 IMS results were obtained were re-examined within 48 hours and the numbers of *E. coli* O157:H7 determined. The methodology for enumeration was described (Pearce *et al.,* 2004a) and used plating of 0.1 ml suspensions of faeces to duplicate CT-SMac plates with the density of *E. coli*

O157:H7 estimated from the bacterial counts after 18-24 hours incubation. Cases of human infection with sorbitol fermenting *E. coli* O157:H- in Scotland and England (Eurosurveillance, 2006) coincided with the last month of abattoir sampling. Therefore, all 83 recta received in this period were screened for sorbitol fermenting *E. coli* O157:H- where the *E. coli* O157 specific IMS beads were cultured onto TBX and CT-SMac agar. Up to ten morphologically distinct sorbitol fermenting colonies were tested by latex agglutination with anti-O157 latex beads (Oxoid, Basingstoke, UK).

6.4. Comparing the detection sensitivity using 1g and 10g faecal sample cultures

This experiment was carried out with the first 100 rectal samples collected to fulfil Objective 1:

1. To test whether there is a difference between 1g and 10g faeces samples in the probability of recovering one or more *Escherichia coli* of serogroups O26, O103, O111, O145 or O157.

2. To evaluate whether there is an improvement in test sensitivity by testing a second sample.

Duplicate 1g and 10g faecal samples from the first 100 recta containing sufficient faeces were therefore tested according to the above methodology for the presence of *E. coli* serogroups O26, O103, O111, O145 or O157.

6.5. PCR detection of *vtx$_1$, vtx$_2$, eae*, EHEC *hlyA* and serogroup specific gene clusters

Multiplex PCR was used to detect genes encoding verocytotoxin (*vtx$_1$*, *vtx$_2$*), intimin (*eae*), enterohaemolysin (*hlyA*) and also O group specific genes (Paton & Paton, 1998; Perelle *et al*., 2004, 2005). The PCR reaction mixes were prepared as described below except the cycling and subsequent electrophoresis conditions used were those described by Paton & Paton (1998). The multiplex assays were separated into two reactions that included in one reaction the *vtx$_1$*:*hlyA* primers and in the second reaction the vtx$_2$:*eae* primers both with the relevant O group specific primers to act as internal controls. 2μL of boiled target was added to the reaction mix. Results were recorded as presence or absence of bands of the expected size (*vtx$_1$*:180bp, *hlyA*:534bp, *vtx$_2$*:255bp, *eae*:384bp, *rfb*E:88bp (O157), *wzx*:135bp (O26), *wzx*:99bp (O103) and *ihpl*:132bp (O145)).

6.6. Statistical analyses and prevalence estimations

Data were analysed using Genstat for Windows v8 (VSN International Ltd, Hemel Hempstead, UK) to fit Generalised Linear Mixed Models, fitting factors such as region and season as fixed effects and factors defining the clustered sampling design as random effects. 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 highly clustered, these models did not always converge to sensible results. In these cases, an alternative approach was used, fitting the random effects model to left censored data (positives only), and weighting the results to reflect the likely proportion of negative lots in the sample. Where the GLMM failed to converge due to uniform effects, the StatXact v6 (Cytel Software Corp, Cambridge, MA, USA) 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. Included in the analyses were the additional factors of "Month" (based on the month sampled) and "Region" (derived from the ear tags recorded). As the samples per month are overly sparse for analysis, a new factor "Season - or Quarter of the Year" was defined from combining monthly data as follows: January to March; April to June; July to September; October to December. Similarly, some of the regions defined by the ear tags have an extremely small associated sample population. Five regions have large numbers of samples and these were retained as unique regions, while the others are grouped together and entitled 'Various'. Where there was evidence of seasonal and age-related differences in the shedding prevalences then the means for each class of animal (categorised by period and age) were combined in a weighted average to give an overall estimate of the mean, where the weights were chosen to reflect the proportion of each class of animal slaughtered in Scotland on average each year.

7. RESULTS

7.1. Comparing the detection sensitivity using 1g and 10g faecal sample cultures

E. coli strains of serogroups O111 and O145 were not recovered from any of the first of 100 faecal samples. The numbers of 1g and 10g samples from which one or more putative *E. coli* O26, O103 or O157 isolates were recovered are given in Tables 3, 4 and 5. Differences in recovery between 1g and 10g samples were evaluated using a two-tailed binomial test and showed no statistical evidence for a difference in recovery of the specific serogroups by size of faecal sample. It is apparent that in each case more target group *E. coli* isolates were recovered from 1g samples than from 10g samples. However, although the testing of 10g faecal samples offers no increase in sensitivity over the use of 1g faecal samples, the testing of duplicate samples does increase the sensitivity of the test procedures at the animal level (Table 6). Therefore, the study protocol was adjusted and the work completed with 1g faecal sampling and testing of all samples in duplicate. This data has not been used to actually determine the sensitivity of the procedure.

Table 3. The numbers of *E. coli* O26 serogroup isolates recovered from duplicate 1g and 10g faecal samples.

		10g samples	
Recovered?		**Yes**	**No**
1g samples	**Yes**	5	4
	No	3	88

P=1.00

Table 4. The numbers of *E. coli* O103 serogroup isolates recovered from duplicate 1g and 10g faecal samples.

Recovered?		10g samples	
		Yes	**No**
1g samples	**Yes**	3	2
	No	1	94

Insufficient positives for a meaningful test

Table 5. The numbers of *E. coli* O157 serogroup isolates recovered from duplicate 1g and 10g faecal samples.

Recovered?		10g samples	
		Yes	**No**
1g samples	**Yes**	4	3
	No	0	93

Insufficient positives for a meaningful test

Table 6. Number of positive isolates by testing a single or second 1g faecal sample.

The cumulative number of faecal samples from which *E. coli* of the appropriate serogroup were recovered is presented in the table below.

	Cumulative number of positive samples	
	First 1g sample	**Second 1g sample**
***E. coli* O26**	5	7
***E. coli* O103**	7	7
***E. coli* O157**	2	3

7.2. Prevalence Estimates

The isolations of each target serogroup by abattoir and sampling date are shown in Table 7. These results are from the 2,086 individual tests irrespective of the replication inherent in the testing. In total, there were 58 isolations of *E. coli* O157, 55 isolations of *E. coli* O26 from TBX media, and 49 from the CT-RMac medium. There were 41 isolations of *E. coli* O103 and 2 isolations of *E. coli* O145, but no isolations of serogroup O111 from any sample.

In Table 8, the same results are shown at animal level where duplicate isolations of a serogroup from replicate samples taken for the same animal are ignored. At animal level from the 1,082 samples there were 37 isolations of *E. coli* O157, 33 isolations of *E. coli* O26 from TBX media and 31 from the CT-RMac. There were 25 isolations of *E. coli* O103 and one isolation of *E. coli* O145.

Irrespective of abattoir the positive isolations of *E. coli* O157:H7 are highly clustered (Tables 7 and 8) with the most extreme clustering arising from 13 positive isolations on a single date in May 2006 from the Greater Glasgow abattoir. There are statistically significant differences in shedding prevalence for *E. coli* O157:H7 in different periods and ages of sheep, as described in greater detail in section 7.4. Therefore the data collected in the survey alone are not sufficient to allow the calculation of a meaningful overall prevalence, as specified in Objective 2. However, using QMS data summarising the ages of animals slaughtered each week in Scotland during 2005 a weighted annual mean was calculated. This showed the mean prevalence of faecal shedding of *E. coli* O157:H7 in sheep at slaughter in Scotland is 3.4% with a 95% confidence interval (0.7%, 9.6%).

Table 7. Numbers of bacterial isolations for each target serogroup from ALL TESTS conducted on samples taken at each abattoir visit.

Sampling		Isolates (by Test)					
	Date	O157	O26	O26ct-rmac	O103	O145	O111
Greater Glasgow	20 July 2005	0	0	2	1	0	0
	09 August 2005	0	0	2	1	2	0
	20 September 2005	0	0	0	2	0	0
	04 October 2005	0	7	0	2	0	0
	08 November 2005	0	2	2	0	0	0
	20 December 2005	0	1	2	0	0	0
	17 January 2006	0	4	0	0	0	0
	21 February 2006	0	1	0	2	0	0
	29 March 2006	0	0	0	0	0	0
	11 April 2006	0	0	0	2	0	0
	23 May 2006	13	0	0	0	0	0
	13 June 2006	0	0	2	1	0	0
		13	15	10	11	2	0
Dumfries & Galloway	19 July 2005	9	0	3	0	0	0
	16 August 2005	5	3	13	3	0	0
	27 September 2005	0	6	1	0	0	0
	10 October 2005	0	2	0	2	0	0
	15 November 2005	12	0	0	1	0	0
	01 December 2005	2	0	0	2	0	0
	10 January 2006	0	0	0	0	0	0
	28 February 2006	0	0	2	0	0	0
	21 March 2006	0	0	0	0	0	0
	25 April 2006	0	0	0	0	0	0
	16 May 2006	2	0	0	0	0	0
	27 June 2006	0	0	0	0	0	0
		30	11	19	8	0	0
Highland	01 August 2005	3	3	4	0	0	0
	30 August 2005	0	3	0	5	0	0
	13 September 2005	0	1	0	0	0	0
	25 October 2005	0	0	0	0	0	0
	01 November 2005	0	0	0	6	0	0
	06 December 2005	0	1	1	0	0	0
	24 January 2006	0	0	0	0	0	0
	07 February 2006	0	2	0	0	0	0
	14 March 2006	0	0	1	0	0	0
	18 April 2006	0	0	0	0	0	0
	09 May 2006	5	0	0	0	0	0
	27 June 2006	0	4	0	2	0	0
		8	14	6	13	0	0
Grampian	26 July 2005	0	3	0	1	0	0
	22 August 2005	2	0	1	3	0	0
	05 September 2005	5	3	9	1	0	0
	17 October 2005	0	4	3	1	0	0
	21 November 2005	0	1	0	3	0	0
	12 December 2005	0	0	0	0	0	0
	30 January 2006	0	1	1	0	0	0
	13 February 2006	0	0	0	0	0	0
	06 March 2006	0	0	0	0	0	0
	03 April 2006	0	3	0	0	0	0
	01 May 2006	0	0	0	0	0	0
	05 June 2006	0	0	0	0	0	0
		7	15	14	9	0	0
Totals		**58**	**55**	**49**	**41**	**2**	**0**

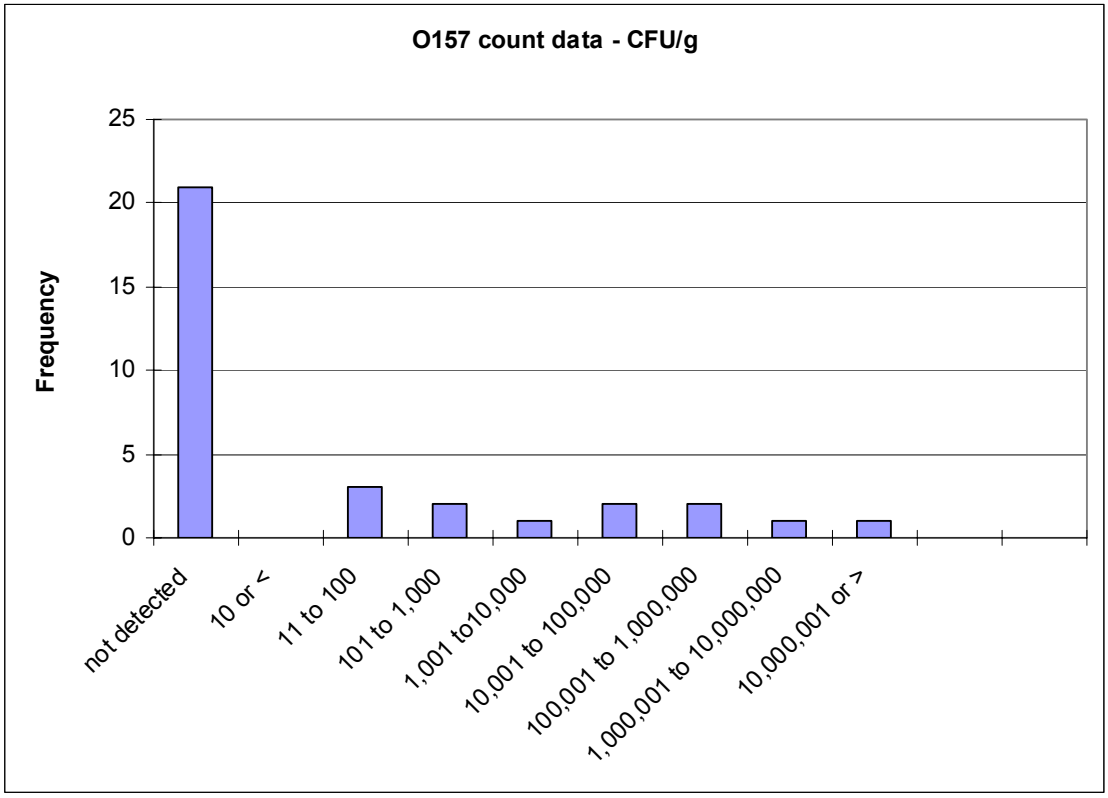
Table 8. Numbers of bacterial isolations for each target serogroup from the ANIMALS sampled at each abattoir visit.

Sampling		Isolates (by Animal)					
	Date	O157	O26	O26ct-rmac	O103	O145	O111
Greater Glasgow	20 July 2005	0	0	2	1	0	0
	09 August 2005	0	0	1	1	1	0
	20 September 2005	0	0	0	1	0	0
	04 October 2005	0	4	0	1	0	0
	08 November 2005	0	1	1	0	0	0
	20 December 2005	0	1	1	0	0	0
	17 January 2006	0	2	0	0	0	0
	21 February 2006	0	1	0	1	0	0
	29 March 2006	0	0	0	0	0	0
	11 April 2006	0	0	0	2	0	0
	23 May 2006	9	0	0	0	0	0
	13 June 2006	0	0	1	1	0	0
		9	9	6	8	1	0
Dumfries & Galloway	19 July 2005	4	0	3	0	0	0
	16 August 2005	4	2	8	1	0	0
	27 September 2005	0	3	1	0	0	0
	10 October 2005	0	1	0	1	0	0
	15 November 2005	8	0	0	1	0	0
	01 December 2005	1	0	0	2	0	0
	10 January 2006	0	0	0	0	0	0
	28 February 2006	0	0	1	0	0	0
	21 March 2006	0	0	0	0	0	0
	25 April 2006	0	0	0	0	0	0
	16 May 2006	1	0	0	0	0	0
	27 June 2006	0	0	0	0	0	0
		18	6	13	5	0	0
Highland	01 August 2005	2	1	2	0	0	0
	30 August 2005	0	1	0	2	0	0
	13 September 2005	0	1	0	0	0	0
	25 October 2005	0	0	0	0	0	0
	01 November 2005	0	0	0	3	0	0
	06 December 2005	0	1	1	0	0	0
	24 January 2006	0	0	0	0	0	0
	07 February 2006	0	2	0	0	0	0
	14 March 2006	0	0	1	0	0	0
	18 April 2006	0	0	0	0	0	0
	09 May 2006	4	0	0	0	0	0
	27 June 2006	0	2	0	1	0	0
		6	8	4	6	0	0
Grampian	26 July 2005	0	2	0	1	0	0
	22 August 2005	1	0	1	1	0	0
	05 September 2005	3	2	4	1	0	0
	17 October 2005	0	2	2	1	0	0
	21 November 2005	0	1	0	2	0	0
	12 December 2005	0	0	0	0	0	0
	30 January 2006	0	1	1	0	0	0
	13 February 2006	0	0	0	0	0	0
	06 March 2006	0	0	0	0	0	0
	03 April 2006	0	2	0	0	0	0
	01 May 2006	0	0	0	0	0	0
	05 June 2006	0	0	0	0	0	0
		4	10	8	6	0	0
Totals		**37**	**33**	**31**	**25**	**1**	**0**

7.3. The frequency and level of excretion of *E. coli* O157:H7 in faeces of sheep presented for slaughter in Scotland

In fulfillment of Objective 3, there were 33 animals positive for *E. coli* O157:H7 that had sufficient faeces present to carry out direct plating and bacterial enumeration as described by Pearce *et. al.* (2004a). In samples from 21 positive animals (64%) the organism was not detected by direct plating, suggesting low level carriage of *E. coli* O157:H7 that was only detectable by IMS. In seven animals the bacterial counts were at 1 x 10^3 CFU/g or above and with one of these samples yielding a count of 1.15 x 10^7 CFU/g of *E. coli* O157:H7 (Figure 1).

Figure 1. Enumeration of *E. coli* O157:H7 in sheep faeces.



7.4. Examination for regional or seasonal variation in the frequency of excretion of *E. coli* O157:H7 in faeces of sheep presented for slaughter in Scotland

Objective 5 was to examine for seasonal or regional variation in the frequency of excretion of *E. coli* O157:H7 in faeces of sheep presented for slaughter in Scotland. Fifty eight *E. coli* O157:H7 isolates were found in samples from 37 animals. Analyses showed a highly statistically significant difference in the mean faecal carriage of *E. coli* O157:H7 in different ages of animal and at different times of year. *E. coli* O157:H7 strains were more commonly isolated from adult and hogg classes of animals than from lambs. Eight positive isolations (4.5%) were made from 179 tests performed on samples from adult sheep, and there were 13 positive (6.2%) from 210 tests on samples from hoggs. However, only 37 positive (2.2%) were identified from 1658 tests performed on lamb samples. Thirty nine tests were performed on samples from animals of unknown age with no positive isolations of *E. coli* O157:H7. At animal level there were 25 lambs (3.0%) positive for *E. coli* O157:H7; seven hoggs (5.6%) and five adult sheep (4.6%). Thus, there was a statistically significant lower level of faecal carriage of *E. coli* O157:H7 strains in lambs compared with adults or hoggs ($p=0.003$).

No isolations of *E. coli* O157:H7 were made during January to March 2006 and this result was significantly lower than found in other quarters of the year ($p<0.001$). The best estimate of the mean *E. coli* O157:H7 excretion frequency of all sheep presented for slaughter in Scotland during January to March was 0% with a 95% confidence interval (0%, 1%). In January to March this absence of serotype O157:H7 was statistically significantly lower than in other months for both the hogg ($p=0.02$) and lamb ($p=0.01$) groups. The observed isolations from adult sheep did not contradict the hypothesis that the mean shedding rate in January to March was lowest, though the observed results did not statistically confirm this hypothesis ($p=0.8$). Thus, the mean *E. coli* O157:H7 excretion frequency for lambs presented for slaughter in Scotland during April to December was 3% with a 95% confidence interval (0.005%, 12.6%). The mean frequency of *E. coli* O157:H7 excretion by adults or hoggs presented for slaughter in Scotland during April to December was 10% with a 95% confidence interval (4%, 17%).

E. coli O157:H7 strains were isolated from 9 sheep sampled at the Greater Glasgow abattoir; from 18 sheep at the Dumfries & Galloway abattoir; 6 sheep in Highland and 4 sheep from Grampian. Thus, there is no evidence of any variation in the faecal carriage of *E. coli* O157:H7 at the four abattoirs ($p=0.42$). Information on the geographic source of the slaughtered animals was available from the ear tags that carry the animals' unique UK flock identifiers. Using this information there were fewer positive results for sheep from Animal Health District 56, which is in the South East of Scotland and administered from the Galashiels Animal Health District Office,

than from the other Scottish districts of 50, 52, 54 and 58. However, there is considerable imbalance in this result. This is because the study was structured at the abattoir level but the submissions to each abattoir involve major differences in the ages and numbers of animals submitted in each season with considerable variation in submissions from the specific regions. In conclusion, the factors of age and season have a greater impact on prevalence than the regional origin of the flock.

7.5. Frequency of virulence determinants in *E. coli* O157:H7

There is no statistically significant evidence of any relationships in vtx_1, vtx_2, *eae* or *hlyA* status in the *E. coli* O157:H7 isolates (Table 9). Three isolates (8% of samples) were vtx_1 positive; 30 (81%) isolates were vtx_2 positive and all isolates (100%) were positive for *eae*. Thirty one isolates (84%) were positive for *hlyA*. All 37 isolates were shown by PCR testing to be serotype O157:H7.

7.6. The frequency of excretion of *E. coli* O103 in faeces of sheep presented for slaughter in Scotland

Isolations of *E. coli* O103 are sparse. There were only 41 faecal samples containing confirmed *E. coli* O103 isolates from 25 animals with eight positive sheep sampled in Greater Glasgow; five in Dumfries and Galloway; six in Highland and six in Grampian (Tables 7 and 8). The estimated mean excretion rate for *E. coli* O103 is 2% with a 95% confidence interval (1%, 3%). Twenty one lambs (2.5%) were positive; one hogg (0.8%) and three adult sheep (2.8%). There is no statistically significant evidence that any of the observed factors or covariates influenced the mean prevalence of *E. coli* O103 shedding.

The duplicate bacterial isolates from each animal were identical when examined by PCR for carriage of the virulence determinants; vtx_1, vtx_2, *eae* or *hlyA*. Of the 25 isolates from individual samples, all were vtx_1 and vtx_2 negative, with seven positive for both *eae* and *hlyA* (Table 10). Thus 0% of isolates were positive for carriage of verocytotoxin genes and 28% of isolates were positive for *eae* and *hlyA.* This result equates to a highly statistically significant ($p<0.001$) positive relationship between carriage of *eae* and *hlyA* genes in serogroup O103 isolates. This carriage of *eae* and *hlyA* virulence genes was confined to serotype O103:H2 isolates; with one other serotype O103:H2 isolate, one serotype O103:H32 isolate and 16 serogroup O103 strains of unknown H type carrying none of the tested virulence genes.

7.7. The frequency of excretion of *E. coli* O111 in faeces of sheep presented for slaughter in Scotland

No *E. coli* O111 isolates were recovered through the 2,086 tests. This corresponds to a mean prevalence of 0% and a 95% confidence interval (0%, 0.3%).

7.8. The frequency of excretion of *E. coli* O145 in faeces of sheep presented for slaughter in Scotland

Two isolates of *E. coli* O145 were recovered from the faecal samples. These were from the rectal and gut swabs from the same lamb, sampled in the Greater Glasgow abattoir in August 2005 (Tables 7 and 8). The best statistical estimate for the faecal carriage of *E. coli* O145 by sheep at slaughter in Scotland is 0.1% with a 95% confidence interval (0.002%, 0.5%). Both isolates were negative for verocytotoxin genes, but positive for *eae* and *hlyA* genes (Table 10) and of an undetermined flagellar serotype.

7.9. The frequency of excretion of *E. coli* O26 in faeces of sheep presented for slaughter in Scotland

Fifty five *E. coli* O26 isolates were recovered on TBX media from a total of 33 animals in 27 lots. The estimated mean excretion rate for *E. coli* O26 by plating to TBX media was 3.4% with a 95% confidence interval (2%, 5%). Nine *E. coli* O26 positive animals were detected in Greater Glasgow; six in Dumfries and Galloway; eight in Highland and 10 in Grampian. In contrast to the *E. coli* O157:H7 isolations, of the 33 animals positive for *E. coli* O26 there were 31 positive isolations from lambs (3.8%); one from a hogg (0.8%), and one from an adult animal (0.9%).

The carriage of vtx_1, vtx_2, *eae* and *hlyA* genes by isolates recovered from the same animal were identical. Thus, examining carriage of virulence genes at animal level revealed three isolates as positive for vtx_1 (9% of samples) (Table 11), none of the 33 isolates were positive for vtx_2 which is 0% of isolates. All 33 isolates from individual animals were positive for the *eae* gene, whilst thirteen isolates were positive for *hlyA* (39% of isolates). Comparing the occurrence of vtx_1 and *hlyA* and analysing the associated contingency table using Fishers Exact test, there is a weak relationship between carriage of vtx_1 and *hlyA* (p-value = 0.052). The serotype of isolates was predominately O26:H11 with only two isolates of undetermined flagellar serotype.

Through plating to rhamnose selective media, 49 *E. coli* O26 isolates were recovered from a total of 31 animals. Six positive animals were detected in Greater Glasgow; 13 in Dumfries and

Galloway; four in Highland and eight in Grampian. Of the 31 positive animals there were 27 lambs (3.3%); two hoggs (1.6%) and two adult animals (1.6%). The estimated mean excretion frequency of *E. coli* O26, by plating to rhamnose selective media, was 4% with a 95% confidence interval (3%, 6%). This result is reported to allow for comparison with the figures for *E. coli* O26 plated to TBX and because there was statistically significant evidence of seasonality (p=0.003). There was little difference between the mean prevalences seen in three quarters of the year, but the mean prevalence in July to September was statistically significantly higher. Three out of the total of six positive animals in Greater Glasgow were positive in July to September; 12 out of 13 in Dumfries and Galloway; two out of four in Highland and five out of the total of eight in Grampian. The estimated mean excretion frequency of *E. coli* O26 for the period October to June, was of a mean prevalence of 0.3% with a 95% confidence interval ($2x10^{-15}$%, 4%); while for July to September we estimate a mean of 8.3% with a 95% confidence interval (1%, 20%). All the isolates were serotype O26:H11.

Comparing the numbers of isolates from animals with vtx_1 or vtx_2 carriage the data suggests that vtx_2 carriage may be positively associated with vtx_1 carriage, but this result is not formally statistically significant (p=0.06). Eight isolates from individual animals were positive for vtx_1 giving 26% of samples and two of these isolates were also positive for vtx_2 yielding 6% of samples. All isolates were positive for *eae* genes *(*100%). 90% of samples were positive for *hlyA* but unlike the isolates from TBX media there is no evidence of any relationship between vtx_1 and *hlyA* status (p=0.39).

In comparing the recovery of *E. coli* O26 from the two different selection media it is noticeable that not only are the positive samples from the two different methods coming from different animals, they also tend to come from different lots. This suggests the true prevalence of *E. coli* O26 is higher than that estimated by either method alone though the confidence intervals of any estimates are wide because of the extremely large between-lot variance. However, there remains statistically significant evidence for a higher prevalence of *E. coli* O26 in the period July to September.

Table 9. *E. coli* serogroup O157 isolates and carriage of genes encoding verocytotoxin (*vtx*), intimin (*eae*) and enterohaemolysin (*hlyA*).

		Virulence determinants				Number of isolates
		vtx$_1$	*vtx*$_2$	*eaeA*	*hlyA*	
***E. coli* O157**		-	-	+	-	6
		+	+	+	+	2
		+	-	+	+	1
		-	+	+	+	28
	Number of determinants	3	30	37	31	**37**
	%	8.1%	81.0%	100%	83.8%	

Table 10. *E. coli* serogroup O103 and O145 isolates and carriage of genes encoding verocytotoxin (*vtx*), intimin (*eae*) and enterohaemolysin (*hlyA*).

		Virulence determinants				Number of isolates
		vtx$_1$	*vtx*$_2$	*eaeA*	*hlyA*	
***E. coli* O103**		-	-	-	-	18
		-	-	+	+	7
	Number of determinants	0	0	7	7	**25**
	%	0%	0%	28.0%	28.0%	
***E. coli* O145**		-	-	+	+	1
	Number of determinants	0	0	1	1	**1**
	%	0%	0%	100%	100%	

Table 11. ***E. coli*** **serogroup O26 isolates recovered from TBX media or rhamnose MacConkey and carriage of genes encoding verocytotoxin (*vtx*), intimin (*eae*) and enterohaemolysin (*hlyA*).**

		Virulence determinants				Number of isolates
		vtx$_1$	*vtx*$_2$	*eaeA*	*hlyA*	
***E. coli* O26 (xTBX)**		-	-	+	-	20
		-	-	+	+	10
		+	-	+	+	3
	Number of determinants	3	0	33	13	**33**
	%	9.1%	0%	100%	39.4%	
***E. coli* O26 (xRMacC)**		-	-	+	-	3
		-	-	+	+	20
		+	-	+	+	6
		+	+	+	+	2
	Number of determinants	8	2	31	28	**31**
	%	25.8%	6.5%	100%	90.3%	

8. DISCUSSION

Comparing the detection sensitivity using 1g and 10g faecal samples justified the use of 1g faecal sampling as previously used in major prevalence and investigative studies of *E. coli* O157 (Besser *et al.,* 1997; Chapman *et al.,* 1997; Mechie *et al*., 1997; Rahn *et al.,* 1997; Pritchard *et al.,* 2000; Wray *et al.,* 2000; Pearce, *et al.,* 2004a & b). It is apparent that more target serogroup isolates were recovered from 1g samples than through 10g sampling though there was no statistically significant evidence of any difference between the two methods. Thus, the 10g faecal samples offered no increase in sensitivity over the use of 1g faecal samples. These findings are supported by a previous study which reported that direct testing from 10g faecal samples offered no significant improvement over 1g samples (Sanderson *et al.,* 1995). Though it is widely considered that 10g sampling should be a more sensitive method, it is paradoxical that as the numbers of *E. coli* O157 per g of faeces are frequently low the testing of 10g samples does not increase the sensitivity of the IMS method. Importantly, the testing of duplicate samples increased the sensitivity of the test procedures. Thus, the work revealed that improvement in test detection can be achieved by increasing the numbers of 1g samples tested per faecal sample, and not by testing larger single amounts such as 10g.

Through the lack of epidemiological work in Scotland there is little epidemiological knowledge of *E. coli* O157:H7 carriage in sheep compared to cattle. This is despite evidence that sheep have acted as sources for human *E. coli* O157:H7 infection in the past (Allison *et al.,* 1997; Licence *et al.,* 2001; Strachan *et al.,* 2001). The results presented here from 2,086 individual tests performed on samples from 1,082 sheep collected at four abattoirs are therefore important in filling this knowledge gap.

Irrespective of abattoir the positive isolations of *E. coli* O157:H7 are highly clustered with the most extreme clustering arising from 13 positive isolations on a single date in May 2006 from the abattoir in Greater Glasgow. Our finding of a major 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 but with a very considerable bias to the mean as 30 of the positive isolations came from a single sampling of 33 sheep in a single flock. This clustering of positive results is consistent with the assumption in the study design that there exists a trimodal distribution of flock prevalences with negative flocks and then positive flocks which include those with a low within flock prevalence and some exhibiting a high within flock prevalence. The detection rate of 3.4% with a 95% confidence interval (0.7%, 9.6%) is consistent with the crude 2.2% prevalence of *E. coli* O157:H7 in sheep faeces reported by Chapman *et al.* (1997) and the 1.7% described by Paiba *et al.* (2002).

The complexities of the sheep industry are apparent from an examination of the submissions of animals to the study abattoirs. In total, 1,082 sheep were sampled. Two hundred and seventy four animals were recorded as received direct from market and amongst these were 40 animals that carried no ear tags and could not be ascribed to any particular source. The remaining 234 animals carried ear tags for 117 individual UK holdings, suggesting that sheep within each market lot had originated on different farms. There were 808 animals received direct from farms that were recorded as originating from 155 individual postcode addresses. Amongst these animals were 87 sheep from one individual farm, but these carried 57 ear tags from separate UK holdings perhaps indicating this farmer was a dealer supplying a particular commercial demand. Excluding these 87 sheep gives 721 sheep from 154 unique farm addresses and with ear tags for 177 UK holdings showing that the majority of animals supplied direct from one farm were from a single flock. The complexity of supply to abattoirs makes difficult the calculation of farm level prevalence. However, the results for the 808 animals received direct from farm include identification of 25 animals positive for verocytotoxigenic *E. coli* O157:H7 and these animals originated from 15 of the 155 unique postal addresses. As carriage of verocytotoxin genes was less frequent amongst the *E. coli* O26 strains, there were only 8 animals positive for VTEC serogroup O26 strains and these animals came from 8 individual farms.

The sampling was inherently biased to farms supplying abattoirs and the study is therefore not a random survey of Scottish sheep farms. Crucially, this bias may have an unknown impact on the results at farm level. However, the above examination is suggestive that the farm level occurrence of verocytotoxin positive *E. coli* O157:H7 or O26 is lower than for cattle where the most recent direct sampling of randomised farms showed 14.7% of farms positive for VTEC of serogroup O157 and 10% for serogroup O26 strains. In part, these results are affected by the higher frequency of verocytotoxin genes in *E. coli* isolates from cattle where 99% of *E. coli* O157:H7 strains were *vtx* positive and 49% of *E. coli* O26 strains. In comparison, the sheep derived isolates showed frequencies for *vtx* carriage of 84% for *E. coli* O157:H7 strains and 17% for *E. coli* O26 strains (combined result from TBX and rhamnose selective media).

In fulfillment of Objective 3 there were 33 animals positive for *E. coli* O157:H7 and with sufficient faeces present to carry out direct plating and bacterial enumeration as described by Pearce *et al*. (2004a). The organism was not detected in many of the positive animals by direct plating. However, we have laboratory results to show that the combined IMS and CT-SMac plating procedure is very sensitive and able to recover reliably fewer than 10^2 CFU/g *E. coli* O157:H7. This suggests that in the majority of positive animals there is low level carriage of *E. coli* O157:H7 that is only detectable by IMS. In seven animals the bacterial counts were at 1 x 10^3 CFU/g or above and with one sample yielding a count of 1.15 x 10^7 CFU/g of *E. coli* O157:H7.

This demonstration of sheep with bacterial counts at 1 x 10^3 CFU/g or above in faeces is consistent with results from cattle where animals exist, termed supershedders, which shed high levels of *E. coli* O157:H7 in faeces. Such animals are considered an important pre-determinant for the clustering of positives within groups (Matthews *et al.,* 2006a & b; Low *et al.,* 2005). There are few other reports of the concentration of *E. coli* O157:H7 in sheep faeces but Ogden *et al.* (2005) in accord with our description of supershedders found 20 of 44 *E. coli* O157 positive sheep shedding at 10^3 CFU/g or above.

Internationally, Scotland has a relatively high rate of *E. coli* O157:H7 related disease in humans with the peak of cases of infection occurring during summer months. However, it was always considered that the achievement of objective 5 requirements by sampling at abattoir would be difficult. Samples were collected in a way that ensured region and season were not confounded, and the data was analysed to explore possible seasonal trends. Analysis of the results of this study showed a highly statistically significant difference in the mean faecal carriage of serogroup *E. coli* O157:H7 at different times of year. No isolations of *E. coli* O157:H7 were made during January to March and the mean faecal shedding prevalence in January to March was thus significantly lower than in other quarters of the year ($p<0.001$). This seasonality is consistent with previous work in a Sheffield abattoir where 17 out of the 22 *E. coli* O157 isolations from sheep were made during the months of June to September inclusive (Chapman *et al.,* 1997) and there was also evidence in a slaughterhouse study of higher faecal shedding by sheep in the period of June, July and August (Paiba *et al.,* 2002). A personal communication from Iain Ogden, University of Aberdeen, told of high numbers of shedding sheep found in Grampian in the summer of 2003.

Intriguingly, the study identified a significant difference in *E. coli* O157:H7 carriage by animal age group. The analyses revealed *E. coli* O157:H7 strains were more commonly isolated from adult and hogg classes of animals than from lambs. There is some concern of the confidence in this result because of the absence of any individual database for sheep in UK and variation in terminologies used in the age classifications at the individual abattoirs where some animals classified as lambs in Highland and Grampian region may have been classed as hoggs elsewhere. Despite this potential bias the analysis showed a statistically significant lower level of faecal carriage of *E. coli* O157:H7 strains in lambs compared with adults or hoggs ($p=0.003$). Interestingly, this is consistent with the findings of a small study of 28 sheep (Ogden *et al.,* 2002). In another small study, Heuvelink *et al.* (1998) found 2 lambs and 2 adults positive for *E. coli* O157 when sampling 52 lambs and 49 ewes in a Dutch abattoir.

In summary, the mean *E. coli* O157:H7 excretion frequency for lambs presented for slaughter in Scotland during April to December was 3.0% with a 95% confidence interval (0.005%, 12.6%). The mean frequency of *E. coli* O157:H7 excretion by adults or hoggs presented for slaughter in Scotland during April to December was 10% with a 95% confidence interval (4%, 17%). The crude detection rate of *E. coli* O157:H7 calculated from the weighted contribution of these means with respect to the relative proportions of adults, hoggs and lambs slaughtered at different times of year allows an estimate of the mean frequency of *E. coli* O157:H7 excretion. The prevalence of *E. coli* O157:H7 carriage by slaughter sheep in Scotland is 3.4% with a 95% confidence interval (0.7%, 9.6%). Overall, our results indicate that the highest risk for carriage of *E. coli* O157:H7 by slaughter sheep in Scotland was during the months of July to September and by adults or hoggs.

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 & Wales (FSA, 2000). The work therefore sought for any regional variation in carriage rates by sheep to complement our knowledge of potential risks to human health. It is notable that isolations were lowest from the Grampian abattoir, which is sited in the region of Scotland with consistently highest rates of human infection. Clearly, it is naïve to suggest that the level of human cases can be linked in any manner to results from limited samplings from a single abattoir. However, the work found no statistical evidence of any variation in the faecal carriage of *E. coli* O157:H7 by abattoir (p=0.42). The analyses also examined for regional variation in results where the flocks of origin were known. These analyses showed no evidence of any geographic variation in the faecal carriage of *E. coli* O157:H7 which is not explained by the imbalance caused by the marked confounding caused by major differences in the ages and numbers of animals submitted to each abattoir. Age and season thus have a greater impact on prevalence than the regional origin of the flock. Crucially, our findings do not support the 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 carriage in sheep in that region.

Interestingly, the isolates of *E. coli* O157:H7 from sheep appear to carry genes for verocytotoxin less frequently than isolates from cattle or humans. The carriage rates of verocytotoxin in isolates of *E. coli* O157:H7 from humans and cattle is generally in the order of 99% (Chapman *et. al.,* 1997; Locking *et. al.,* 2006). This study identified 84% of sheep isolates as vtx_1 and/or vtx_2 positive with vtx_2 being most frequent and found in 81% isolates. This finding is similar to previous results from sheep (Chapman *et. al.,* 1997; Paiba *et. al.,* 2002). It therefore appears that there is a greater variation in *E. coli* O157:H7 strains from sheep compared to cattle and that

these verocytotoxin negative strains are not found causing illness in humans where strains carrying genes for *vtx*, *eae* and *hlyA* make up 99% of isolations.

In Continental Europe, the most common non-O157 VTEC 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 and Tozzi, 1998). In this study, the estimated mean excretion rate in slaughter sheep for *E. coli* O103 is 2%. This isolation rate compares to the 2.7% weighted mean prevalence of *E. coli* O103 seen in beef cattle in Scotland (Pearce *et al.,* 2006)**.** There is no statistically significant evidence that animal age, season or region affected the prevalence of *E. coli* O103 shedding. Of the 25 isolates from individual animals, all were vtx_1 and vtx_2 negative, with seven isolates positive for both *eae* and *hlyA*. Thus, the virulence gene carriage is similar to that found in strains isolated from cattle (Pearce *et al.,* 2006) and is an indication that VTEC of serogroup O103 are rare from both sources. Previously, Beutin *et al.* (2005) have shown two clonal divisions of *E. coli* O103:H2 strains describing as enteropathogenic those with *eae* of the epsilon variant and *E. coli* O103:H2 strains with beta *eae* variant being enterotoxigenic and also carrying *vtx* genes. The *eae* variants of the *E. coli* O103:H2 isolates described in our work are unknown but it would be of interest to confirm the gene variants so as to ascribe the seven isolates to the EPEC or EHEC lineages.

In Norway, *E. coli* O103 strains associated with human illness have been reported (Urdahl, *et al*., 2002), and more recently (Schimmer, 2006). The first reported isolate was of unknown flagellar type and carried vtx_1 and *eae*, whereas the second cases were caused by *E. coli* O103:H25 strains that were mostly vtx_2 and eae positive. Bacterial culture of faeces from sheep, thought to be linked with the first case, revealed very few VTEC O103 isolates which is consistent with our findings. The authors reported considerable numbers of *vtx* negative, *eae* positive *E. coli* O103 strains which would be consistent with the strains described in this study. In the second report the verocytotoxin positive outbreak strain was isolated from mutton (Schimmer, 2006).

A highly statistically significant ($p<0.001$) positive relationship between carriage of *eae* and *hlyA* genes was demonstrated in *E. coli* O103 isolates. This carriage of *eae* and *hlyA* virulence genes was confined to serotype O103:H2 isolates. The very strong association found between carriage of *eae* and *hlyA* genes has previously been described (Boerlin *et al*., 1999; Blanco *et al*., 2004). Whilst both *eae* and *hlyA* encode important virulence determinants the isolates from sheep that are *eae* gene positive but *vtx* negative are generally regarded as atypical enteropathogenic *E. coli* (EPEC) and may be less likely than VTEC to cause human disease (Ramachandran, *et al*., 2003).

Two isolates of *E. coli* O145 were recovered from a single lamb sampled in the Greater Glasgow abattoir in August 2005. Both isolates were negative for verocytotoxin genes, but positive for *eae* and *hlyA* genes and of an undetermined flagellar serotype. We have also identified a low shedding prevalence of verocytotoxigenic *E. coli* O145 in cattle (Jenkins *et al*., 2002; Jenkins *et al*., 2003; Pearce *et al*., 2004b). No *E. coli* O111 isolates were recovered from the 2,086 samples. 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. In summary, the study found very limited carriage of verocytotoxin positive strains amongst the potential VTEC serogroups of O103, O111 or O145 in 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 VTEC strains isolated from amongst 401 random *E. coli* isolates recovered from sheep at slaughter in England and Wales.

Fifty five *E. coli* O26 isolates were recovered on TBX media from a total of 33 animals in 27 lots. The estimated mean excretion rate for *E. coli* O26 by plating to TBX media was 3.4% with a 95% confidence interval (2%, 5%). Plating to rhamnose selective media has been suggested as assisting in the identification of VTEC positive strains (Wieler *et al.,* 1995) and forty nine *E. coli* O26 isolates were recovered from a total of 31 animals. The estimated mean excretion frequency of *E. coli* O26, by plating to rhamnose selective media, was 4% with a 95% confidence interval (3%, 6%). Thus, there is overlap of the confidence intervals for the prevalence estimates from using the two media. However, when comparing the recovery of *E. coli* O26 from the two different selection media it is noticeable that not only are the positive samples from the two different methods coming from different animals, they also tend to come from different lots. This suggests the true prevalence is higher than that estimated by either method alone. There is evidence from the PCR characterisations that the two plating media were identifying different strains within *E. coli* O26 serogroup but it may also be that the result is a consequence of a low sensitivity of IMS and selective plating for *E. coli* O26 recovery (Hall *et al.,* 2006). In comparison, to the 3.4% with a 95% confidence interval (2%, 5%) found in sheep using isolation on TBX media alone, the weighted mean prevalence of serogroup O26 in cattle faeces in Scotland was 4.6% (3%, 6.6%).

The results from the rhamnose selective media exhibited evidence of seasonality (p=0.003) where the mean prevalence in July to September was statistically significantly higher than in the other quarters. The estimated mean excretion frequency of *E. coli* O26 for the remainder of the year was of a mean prevalence of 0.3% with a 95% confidence interval ($2x10^{-15}$%, 4%); while for July to September we estimate a mean of 8.3% with a 95% confidence interval (1%, 20%).

From the rhamnose selective medium all the isolates from sheep were serotype O26:H11 and predominately from the TBX medium with only two isolates of indeterminate flagellar serotype. The carriage of *vtx*$_1$, *vtx*$_2$, *eae* and *hlyA* genes by isolates recovered from the same animal were identical. The use of rhamnose selective media identified more VTEC strains with eight isolates from individual animals positive for *vtx*$_1$ giving 26% of samples with a 95% confidence interval of (12%, 45%) and two of these isolates also positive for *vtx*$_2$ yielding 6% of samples with a 95% confidence interval (0.8%, 21%). In contrast, from TBX media only three isolates were positive for *vtx*$_1$, which is 9% of samples with a 95% confidence interval (2%, 24%). None of the 33 isolates were positive for *vtx*$_2$ which is 0% of isolates with a 95% confidence interval (0%, 11%). As with our previous findings for *E. coli* O26 isolates from cattle there was no carriage of *vtx*$_2$ genes without *vtx*$_1$. Irrespective of the isolation medium, the carriage rates of *vtx* genes are lower than the 49% of *E. coli* O26 that were *vtx* gene positive from cattle (Pearce *et al.,* 2006). Therefore, in Scotland sheep appear less likely than cattle to be a source of verocytotoxigenic *E. coli* O26 strains.

From both rhamnose selective and TBX media all *E. coli* O26 isolates from individual animals were positive for the *eae* gene which is a carriage of 100%. From TBX plates there were fewer isolates *hlyA* positive than from the rhamnose selective medium and thus, the rhamnose selective medium tends to identify greater numbers of *vtx* positive and eae, *hlyA* positive strains of *E. coli* O26. These strains may be more likely to cause human infections. *E. coli* O26 is the most common non-O157 VTEC found in human disease in Spain (Blanco *et. al*., 2004). The authors also refer to it as the first or second most common type in human non-O157 infection in Germany, Belgium, Denmark, Finland, Canada, US and Japan (Blanco *et. al*., 2004). In the UK, *E. coli* O26 infections are uncommon but have been recognised (Smith, *et. al*., 2001; Willshaw *et. al*., 2001; Evans *et. al*., 2002) and strains with *vtx*$_1$, *eae* and *hlyA* have been identified as particularly likely to cause severe human infections (Willshaw *et. al*., 2001; Blanco, *et. al*., 2004). The Scottish *E. coli* Reference Laboratory (SERL) identified four Scottish clinical cases of *E. coli* O26 in people during a six-week period in June and July 2003. During 2006, SERL identified 18 isolates of non-O157 VTEC from human faeces submitted in line with Task Force recommendations. These persons included five infected with *E. coli* of serogroup O26, two with serogroup O111 and one with serogroup O103. Of the five *E. coli* O26 isolates, two possessed both *vtx*$_1$ and *vtx*$_2$ genes. The other three isolates possessed *vtx*$_2$ only. (*Lesley Allison personal communication*). In the same 2006 period, SERL reported a provisional total of 244 culture positive cases of *E. coli* O157 infection (Locking *et. al.,* 2007).

In this study, there were 31 out of 38 isolates of *E. coli* O157:H7 carrying *vtx*, *eae* and *hlyA* genes. For *E. coli* O26 there were 11 out of 64 isolates carrying virulence genes giving them the

potential to cause human infections. Despite the apparent carriage of *E. coli* O26 strains in sheep in Scotland that are potentially virulent for humans it remains unclear why the numbers of *E. coli* O26 infections observed in humans in Scotland are low (Locking *et. al.,* 2007).

We have provided a benchmark for shedding of VTEC and evidence that VTEC strains of serogroups O103, O111 or O145 are of low prevalence or absent from sheep at slaughter in Scotland. Despite the carriage of *E. coli* O26 strains in sheep in Scotland that are apparently potentially virulent for humans the results of enhanced surveillance continues to show *E. coli* O157:H7 strains as the greatest threat to human health. The highest risk for carriage of *E. coli* O157:H7 by slaughter sheep in Scotland was during the months of July to September and by sheep that were adults or hoggs. Crucially, our findings do not support the 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. Evidence was produced that supershedders for *E. coli* O157:H7 exist in sheep populations and these animals are presumed to be the reason for the marked clustering of positives within groups. Such concentrations of infection are considered likely to pose a risk for human infections.

9. CONCLUSIONS

Objective 1 was fulfilled and identified that improvement in IMS test detection can be achieved by increasing the numbers of 1g samples tested per faecal sample. The study was therefore completed with testing of all samples in duplicate and provides a benchmark for shedding of VTEC organisms by sheep at slaughter in Scotland.

In fulfillment of Objective 2 evidence was produced for a greater variation in *E. coli* O157:H7 strains from sheep compared to cattle and that the verocytotoxin negative strains are not found in humans where strains carrying genes for *vtx*, *eae* and *hlyA* make up 99% of isolations. Potentially virulent strains of *E. coli* O157:H7 were found in sheep and irrespective of abattoir the positive isolations of *E. coli* O157:H7 are highly clustered. The mean prevalence of faecal shedding of *E. coli* O157:H7 in sheep at slaughter in Scotland is 3.4% with a 95% confidence interval (0.7%, 9.6%). The highest risk for carriage of *E. coli* O157:H7 by slaughter sheep in Scotland was during the months of July to September and by sheep that were adults or hoggs. There is no evidence of any variation in the faecal carriage of *E. coli* O157:H7 at the four abattoirs (p=0.42). Crucially, our findings do not support the 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. The factors of age and season have a greater impact on prevalence in sheep than the regional origin of the flock.

Sheep with bacterial counts at 1 x 10^3 CFU/g or above in faeces were identified and are consistent with results from cattle where animals exist, termed supershedders, which shed high levels of *E. coli* O157:H7 in faeces. Their existence is presumed to be the reason for the marked clustering of *E. coli* O157:H7 positives within groups of sheep.

VTEC strains of serogroups O103, O111 or O145 are of low prevalence or absent. Despite the carriage of *E. coli* O26 strains in Scottish sheep that are VTEC positive and apparently potentially virulent for humans, the results of enhanced surveillance continues to show *E. coli* O157:H7 strains as the greatest threat to human health.

10. RECOMMENDATIONS

1. The occurrence of higher level faecal carriage of *E. coli* O157:H7 can be examined to determine if there is evidence for the clusters of positive animals being attributable to the presence of supershedders. This should clarify if higher transmission rates are attributable to these animals.

2. The animals with high level faecal carriage of *E. coli* O157:H7 could be identified and examined to determine if there is evidence for rectal colonisation.

3. The absence of serogroup O111 strains from Scottish sheep is remarkable and further monitoring is recommended.

4. Possibly because of difficulties in routinely identifying non-O157 VTEC there has been no actual epidemiological linkage between human cases of serogroup O26 disease and animal sources of infection. This is in contrast to *E. coli* O157:H7 infection and an epidemiological follow-up to clinical cases of *E. coli* O26 is recommended with further typing of isolates.

5. The *E. coli* O26 strains recovered in this study should be made available for pulsed-field gel electrophoresis to determine the relatedness of strains to isolates recovered from humans and cattle in Scotland.

11. PUBLICATIONS AND PRESENTATIONS

11.1. Peer-reviewed Publications

None. However, referred papers are in preparation and should be submitted shortly to peer-review.

11.2. Conference Proceedings

Evans, J; Varo, A; Stevenson, H; Knight, H I; McKendrick, I J; Naylor, S W; Gunn, G J; Low, J C (2006). Prevalence of faecal shedding of Verocytotoxigenic *Escherichia coli* serogroups O26, O103, O111 and O145 in Scottish sheep. *Verocytotoxigenic Escherichia coli Conference: 6th International Symposium on Shiga Toxin (Verocytotoxin) - Producing E. coli Infections.* October, Melbourne, Australia. P14.1.02.

11.3. Oral Presentations

Low, J C (2006). The epidemiology of VTEC in ruminants. *Health Protection Scotland.* 12th March 2006, SERL Western General, Edinburgh, UK.

Evans, J SAC Inverness. Field work with VTEC in cattle and sheep. Scottish VTEC meeting. Friday 3rd March 2006. Medical School, Foresterhill, Aberdeen.

Low, J C (2006). The epidemiology of VTEC in sheep at slaughter. *FSAS Research Day.* 17th May 2007, Dynamic Earth, Edinburgh, UK.

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