

Changing Patterns in Bovine *E. coli* O157 Shedding in Scotland

Pearce, M.¹; Mellor, D.²; Ternent, H.²; McKendrick, I.³; Smith, A.¹; Knight, H.⁴; Woolhouse, M.¹; Low, C.⁴; Syngé, B.⁴; Gunn, G.⁴

¹Centre for Tropical Veterinary Medicine, University of Edinburgh, Roslin, Midlothian EH25 9RG, UK; ²Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, 464 Bearsden Rd, Glasgow G61 1QH, UK; ³Biomathematics and Statistics Scotland (BIOSS), James Clerk Maxwell Building, Kings Buildings, Edinburgh EH9 3JZ; ⁴Animal Health Group, Scottish Agricultural College, West Mains Rd, Edinburgh EH9 3JG, UK.

Abstract

Escherichia coli O157 is an important infectious disease of humans, causing disease ranging from mild diarrhoea to fatal haemorrhagic uraemic syndrome, and cattle are regarded as a major reservoir and amplifying host. Scotland has one of the highest rates of human *E. coli* O157 infection in the world. Two extensive surveys of Scottish beef cattle herds have been conducted: one from 1998 to 2000, the second from 2002 to 2004. In both surveys, probability samples were selected from the entire population of Scottish farms with beef cattle. On each farm, faecal pats were sampled using a prescribed sampling plan. Prevalence overall and among temporal and spatial subsets were analysed using bootstrap methods. At the farm level, changes to the prevalence of shedding between surveys were modest. The overall prevalence fell from 21.9% to 18.7% and there were small decreases in prevalence across all seasons and regions of Scotland with the exception of summer, and the Highlands and South West. At the pat level, changes in the prevalence of shedding were greater. The overall prevalence fell from 8.9% to 3.9%, and halved in all seasons. Prevalence decreased substantially in all regions except the South West where there was a small increase. The principal phage types shed in both surveys were PTs 2, 4, 21/28 and 32, and the majority of strains isolated carried genes encoding the virulence factors verocytotoxin and intimin.

Introduction

Escherichia coli O157 is an important infectious disease of humans, causing disease ranging from mild diarrhoea to fatal haemorrhagic uraemic syndrome (Karmali, 1989), and cattle are regarded as a major reservoir and amplifying host (Armstrong *et al.*, 1996). Scotland has had one of the highest rates of human *E. coli* O157 infection in the world (Locking *et al.*, 2006), and following major outbreaks, SEERAD funded a national survey of cattle to determine the importance of cattle as a source of O157 shedding. This was followed four years later by a comparative national survey conducted by the Wellcome funded IPRAVE research consortium.

Material and Methods

Two surveys of Scottish store and finishing cattle were conducted: the first from 1998 to 2000, the second from February 2002 to January 2004. Both surveys preferentially sampled store or finishing cattle closest to sale or slaughter. If such groups did not exist on a particular farm, one or more mixed groups with store or finishing cattle closest to sale or slaughter were sampled.

In the first (SEERAD) survey, 952 farms were randomly selected and surveyed. The number of faecal pats sampled from each group was determined from the number of cattle in the group using a prescribed sampling schedule. For each group, sufficient numbers of faecal pats were sampled to ensure an 80% chance of sampling at least one positive pat assuming there was a prevalence of $\geq 2\%$ shedding within the group.

In the second (IPRAVE) survey, the sampling frame comprised 925 farms sampled in the first survey whose owner or manager consented to an additional visit. Within this sampling frame there

were insufficient farms to adequately represent two state animal health divisions (AHD) so additional farms for these two AHDs, Highland and Islands, were recruited by random selection. A multistage sampling plan was used to select faecal pats. Farms were stratified by AHD and strata sampled systematically to ensure similar numbers of farms were sampled from each stratum. At each sampling timepoint, a cluster of three farms was selected randomly. Each cluster was stratified by farm, and store and finishing cattle on each farm identified and stratified by management group. The number of faecal pats to be sampled from each group was determined from the number of cattle in the group using a prescribed sampling schedule. For each group, sufficient numbers of faecal pats were sampled to ensure a 90% chance of sampling at least one positive pat assuming there was a prevalence of $\geq 2\%$ shedding within the group.

Faecal pats were sampled without replacement, and all samples were refrigerated at 5°C as soon as possible after collection and always processed within 48 hours of collection. Within 48 hours of sampling, one gram of faeces from each sample was tested for the presence of *E. coli* O157 as previously described (Pearce *et al.*, 2004)

Prevalence Estimates

For both surveys, distributions of the overall prevalence of shedding, overall phage type specific shedding, and shedding within AHD or seasonal subsets were generated using bootstrap sampling with 10,000 iterations. The same number of farms or pats sampled in the original surveys was sampled using the sampling procedure used in the original surveys, but with replacement. The mean (PE_{mean}), median (PE_{median}) and upper and lower confidence limits (PE_{ucl} , PE_{lcl}) of shedding were derived from the respective bootstrap distributions.

Comparison of Animal Health Districts

For both the SEERAD and IPRAVE surveys, a null distribution of differences between AHDs (A) was generated, using bootstrap sampling with 10,000 iterations. In each iteration, for each AHD, farms or pats were sampled from pooled data arising from the original surveys; the same number of farms or pats sampled in the original surveys was sampled using the sampling procedure used in the original surveys, but with replacement, and an estimate of prevalence returned for each AHD. For each iteration, the maximum pairwise difference between the prevalence estimates for the six AHDs was returned (Diff_{max}). Pairwise differences between PE_{mean} for the six AHDs were compared with the distribution of Diff_{max} , to determine if AHDs had a significant impact on prevalence of shedding.

Comparison of SEERAD and IPRAVE Survey Prevalence Estimates

A null distribution of differences in prevalence between SEERAD and IPRAVE surveys (B) was generated using bootstrap sampling with 10,000 iterations. Farms from the SEERAD survey and farm clusters from the IPRAVE survey were pooled as sampling units. In each iteration, a total of sampling units equivalent to the number of farms sampled in the SEERAD survey, and the number of farm clusters sampled in the IPRAVE survey respectively was selected, with replacement. If a farm cluster was selected, one farm was selected at random from the cluster. In each iteration, the proportion of farms or pats positive was calculated and the difference between the prevalence estimates for the SEERAD and IPRAVE surveys ($\text{Diff}_{\text{null}}$) was determined.

A distribution of IPRAVE prevalence estimates using a sampling process equivalent to simple random sampling was generated using bootstrap sampling with 10,000 iterations. In each iteration, a farm was randomly selected from each of the clusters sampled in the original IPRAVE survey, and the IPRAVE prevalence was estimated. In each iteration, the median SEERAD prevalence estimate (PE_{median}) was subtracted from the IPRAVE prevalence estimate to give a distribution of differences between SEERAD and IPRAVE prevalence estimates using equivalent sampling plans ($\text{Diff}_{\text{prev}}$).

The differences in the null distribution (B) were ranked and compared with $\text{Diff}_{\text{prev}}$ to give the probability of there being a difference in prevalence between the SEERAD and IPRAVE surveys.

Results

14,849 faecal pats across 952 farms were sampled in the SEERAD study and 12,963 pats across 481 farms in the IPRAVE study. A total of 1,296 *E. coli* O157 strains were isolated from the SEERAD study and 1,269 strains in the IPRAVE study. Farm and pat level prevalence estimates for the two surveys are given in Tables 1 and 2.

Table 1. Farm Level prevalence of bovine *E. coli* O157 shedding in the SEERAD and IPRAVE surveys.

| Category | Prevalence (lower, upper 95% confidence limits) | | P value |
|----------------------------------|---|----------------------|---------|
| | SEERAD (%) | IPRAVE (%) | |
| All categories | 21.89 (19.54, 24.26) | 18.68 (14.92, 22.55) | 0.3514 |
| By season | | | |
| Spring | 22.10 (17.13, 27.41) | 17.52 (11.61, 23.48) | 0.5224 |
| Summer | 22.91 (18.22, 27.57) | 24.77 (17.36, 32.23) | 0.7824 |
| Autumn | 26.57 (22.52, 30.63) | 20.63 (15.75, 25.78) | 0.4970 |
| Winter | 14.88 (11.79, 18.46) | 9.77 (6.50, 13.01) | 0.8076 |
| By animal health district | | | |
| Highland | 16.36 (10.87, 21.74) | 18.32 (8.54, 28.05) | 0.7804 |
| North East | 24.72 (20.09, 28.97) | 18.38 (9.88, 27.16) | 0.4554 |
| Central | 24.89 (20.54, 29.73) | 17.30 (12.00, 22.67) | 0.3460 |
| South West | 18.84 (13.52, 25.00) | 24.65 (16.05, 32.10) | 0.3228 |
| South East | 25.23 (20.00, 30.37) | 23.53 (18.29, 29.11) | 0.7264 |
| Islands | 17.10 (10.98, 23.17) | 9.85 (6.02, 13.75) | 0.6070 |
| By phage type | | | |
| PT2 | 3.26 (2.42, 4.20) | 1.66 (0.84, 2.32) | 0.3438 |
| PT4 | 1.27 (0.74, 1.79) | 0.63 (0.21, 1.24) | 0.5308 |
| PT8 | 1.05 (0.53, 1.68) | 1.87 (0.84, 2.90) | 0.3596 |
| PT21/28 | 13.46 (11.66, 15.34) | 10.38 (7.90, 12.92) | 0.3226 |
| PT32 | 3.06 (2.15, 3.99) | 5.4 (3.75, 7.13) | 0.1064 |
| PT34 | n.a. | 0.21 (0.00, 0.62) | n.a. |

The farm level prevalence decreased from 21.9% to 18.7% between the two surveys although this was not statistically significant. In spring, autumn and winter, farm level prevalence of shedding decreased 21–33% and in summer it increased 8%; in four AHDs farm level prevalence decreased 7–43%, but increased 12% in Highland and 30% in South West. None of the changes among the seasons or AHDs was statistically significant. Among the principal PTs, the farm level prevalence of PTs 2, 4 and 21/28 decreased 23–51% but the prevalence of PT 32 increased 54%. However, none of the changes in the prevalence of PTs was significant.

The pat level prevalence of *E. coli* O157 more than halved from 8.9% to 3.9% ($P=0.0090$). Over the same period, there were falls in the prevalence of shedding in all seasons by 40–50% though none were statistically significant. In five of the six Scottish AHDs there were falls in the prevalence of

shedding, the highest being in North East with a fall of 76%, but in South West the prevalence rose 32%. None of these changes was statistically significant however. Among the major phage types, there was a substantial decrease in the prevalence of PT21/28 shedding from 5.2% to 1.9% ($P=0.0258$). PT21/28 was the dominant phage type isolated in both studies representing 56.3% of strains in the SEERAD study and 50.8% of strains in the IPRAVE study. The prevalence of the remaining major phage types found in these two surveys, PTs 2, 4 and 32, declined by 68%, 93% and 32% respectively though none was statistically significant.

Table 1. Pat level prevalence of bovine *E. coli* O157 shedding in the SEERAD and IPRAVE surveys.

| Category | Prevalence (lower, upper 95% confidence limits) | | P-value |
|----------------------------------|---|--------------------|---------|
| | SEERAD (%) | IPRAVE (%) | |
| All categories | 8.89 (7.46, 10.55) | 3.93 (2.91, 5.12) | 0.0090 |
| By season | | | |
| Spring | 10.34 (6.79, 14.36) | 4.38 (2.60, 6.41) | 0.1404 |
| Summer | 8.39 (5.25, 11.66) | 3.83 (1.94, 5.72) | 0.2666 |
| Autumn | 8.58 (5.93, 11.34) | 4.42 (2.20, 6.90) | 0.2420 |
| Winter | 7.40 (4.79, 10.37) | 3.02 (1.03, 5.22) | 0.3668 |
| By animal health district | | | |
| Highland | 9.56 (3.94, 15.39) | 2.30 (1.14, 3.59) | 0.1572 |
| North East | 11.49 (7.92, 14.98) | 2.28 (1.13, 3.64) | 0.0730 |
| Central | 9.31 (6.72, 12.00) | 3.32 (1.65, 5.20) | 0.1626 |
| South West | 5.05 (2.23, 8.32) | 6.70 (3.60, 10.21) | 0.5796 |
| South East | 10.62 (7.03, 14.51) | 5.19 (1.87, 9.73) | 0.2762 |
| Islands | 6.19 (2.08, 10.95) | 4.21 (1.28, 7.50) | 0.6890 |
| By phage type | | | |
| PT2 | 1.32 (0.76, 1.94) | 0.42 (0.01, 0.79) | 0.2064 |
| PT4 | 0.57 (0.24, 0.94) | 0.04 (0.00, 0.09) | 0.2354 |
| PT8 | 0.35 (0.10, 0.65) | 0.41 (0.06, 0.98) | 0.9262 |
| PT21/28 | 5.19 (3.93, 6.72) | 1.94 (1.30, 2.62) | 0.0258 |
| PT32 | 1.00 (0.62, 1.40) | 0.66 (0.26, 1.15) | 0.6370 |
| PT34 | n.a. | 0.19 (0.00, 0.58) | n.a. |

Among strains isolated during the SEERAD study, 0.2%, 94.9% and 4.9% possessed genes encoding the virulence factors VT1 only, VT2 only and VT1+VT2 respectively. Among strains isolated during the IPRAVE study, 0.8%, 89.7% and 8.9% possessed genes encoding VT1 only, VT2 only and VT1+VT2 respectively. All strains isolated from both studies possessed *eae*, the gene encoding the virulence factor intimin.

Discussion

These are the only systematic national surveys of bovine *E. coli* O157 shedding reported and provide a valuable opportunity to examine changes in the patterns of shedding and strain characteristics. Knowledge of the level of bovine shedding is important as cattle are regarded as a major potential source of *E. coli* O157 for human infection, either from food or water contaminated by bovine faeces or through direct contact of cattle (Locking *et al.*, 2001).

Over the 4-year period between the surveys there was a substantial decrease in the proportion of cattle shedding *E. coli* O157, but the proportion of farms with shedding cattle appears to have decreased much less, if at all. A similar dissociation between the prevalence at the pat and farm level has been described for non-O157 strains of *E. coli* too (Pearce *et al.*, 2006). It is possible that at the farm level, *E. coli* O157 shedding may stop or remain undetectable in many cattle but still remain on the farm, possibly attaining a state of equilibrium, and there are reports of extended *E. coli* O157 activity on farms (Vali *et al.*, 2005). These points have important implications for control programmes and the assessment of their efficacy. It remains to be seen whether this decline in the prevalence of *E. coli* O157 shedding continues, but there are precedents among other members of the Enterobacteriaceae family e.g. *Salmonella* to suggest that it is a possibility.

With some exceptions, there was a general trend downwards in the prevalence of shedding at both the farm and pat level across seasons, AHDs and phage type. However, despite the large numbers of farms and pats sampled in the two studies, there was insufficient statistical power to achieve statistical significance in most pairwise comparisons between the survey results.

The decline in the prevalence of shedding appears to have been driven by a fall in the shedding of PT21/28. Interestingly, this has been the major PT associated with human infections in Scotland which also declined in the interval between the two studies of bovine shedding (Locking M. *et al.*, 2006). This provides further evidence of a link between bovine shedding and human infection.

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