

Longitudinal studies of *E.coli* O157 shedding in dairy cattle; an examination of microbial counts and strain diversity.

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Abstract

Five dairy farms were visited monthly for at least 12 months and fresh bovine faecal samples were taken from each of 5 management groups: unweaned and weaned calves, heifers, lactating and dry cows. Samples were tested for the presence and numbers of *E.coli* O157 via direct spiral plating and enrichment followed by immunomagnetic separation (IMS). No consistent seasonal pattern was observed across farms although the prevalence and numbers shed varied markedly within farms over time. On four farms the weaned youngstock had the highest prevalence of *E.coli* O157 although, on average, the unweaned calves shed more *E.coli* O157 per gram. Strains of *E.coli* O157 were highly conserved within farms and neighbouring farms had indistinguishable strains.

Introduction

Cattle are widely regarded as important reservoirs of infection for both food and environmental pathways. Previous longitudinal studies have concluded that shedding is usually of a transient nature, with weaned calves having the highest prevalence within the farm environment. A longitudinal study of five dairy farms was undertaken to explore the relationship between age, management group and shedding and inform mathematical models of *E.coli* O157 transmission in dairy herds.

Materials and Methods

Systematic sampling identified five farms with cattle shedding *E.coli* O157. The farms were sampled every four weeks over a 12-20 month period. For routine sampling, faecal samples were taken from the pen floor after cows were observed defecating. An enumeration method for *E.coli* O157 based on spiral plating of faecal broths onto HarlequinTM SMAC-BCIG agar was developed to further describe patterns of shedding. Faecal samples were diluted in buffered peptone water and vancomycin (BPW +V) and a sub sample directly plated, for enumeration purposes, whilst the remaining broth was enriched overnight and subjected to IMS to give more sensitive presence / absence data. Multilevel risk factors were recorded on farm visits.

Results

No obvious consistent seasonal pattern was identified although some variation within farms was evident. Figure 1 illustrates the temporal patterns in shedding for Farm 1 over 18 months for 4 of the management groups. Prevalence estimates over the sampling period varied greatly, between 0 - 42% for within-herd and between 0 - 100% for within management group. Table 1 indicates the overall farm prevalence for

E. coli O157 positive samples over the first 9 months of the study. This is broken down into samples that had concentrations of *E. coli* O157 above 200cfu/g. Farm A and Farm D had the highest prevalence of the farms in the study and these were contiguous premises. Using RFLP-PFGE indistinguishable strains of *E. coli* O157 were recovered from the contiguous farms as can be seen from figure 3. On four farms the weaned group had the highest prevalence but unweaned calves had higher counts per gram of *E. coli* O157 present (Fig 2.).

| Farm No. | No.+ve samples | Total No. of samples | % +ve sampes | No. samples with countable O157 |
|----------|----------------|----------------------|--------------|---------------------------------|
| A | 93 | 821 | 11 | 55 |
| B | 3 | 265 | 1 | 0 |
| C | 21 | 460 | 5 | 4 |
| D | 36 | 243 | 15 | 9 |
| E | 18 | 269 | 7 | 9 |

Table 1. *E. coli* O157 results from longitudinal sampling on 5 farms

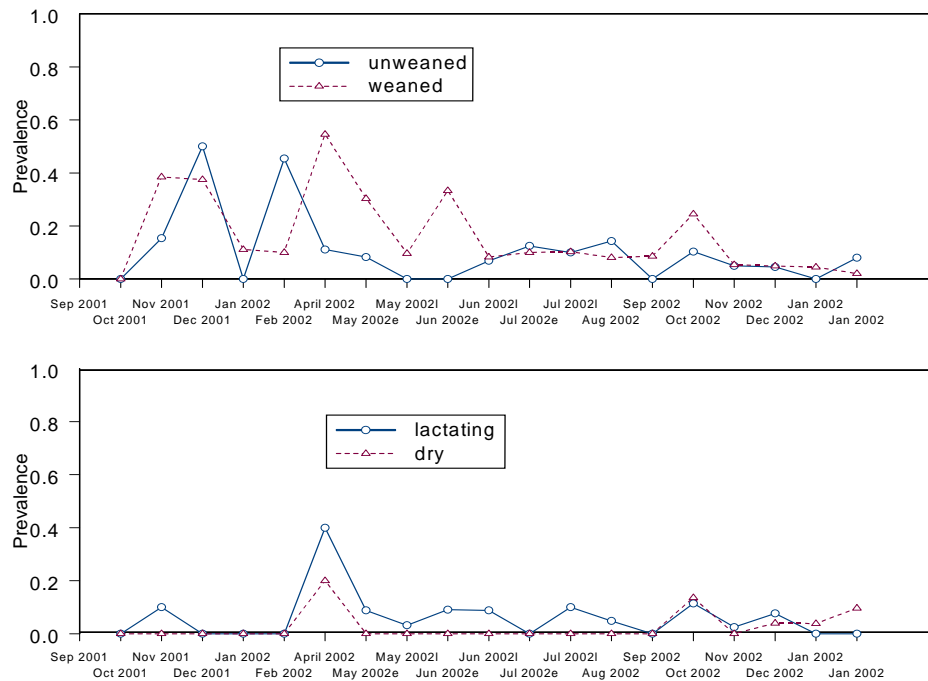


Figure 1. Seasonal patterns of *E. coli* O157 shedding for different management groups on Farm 1 over 18 months.

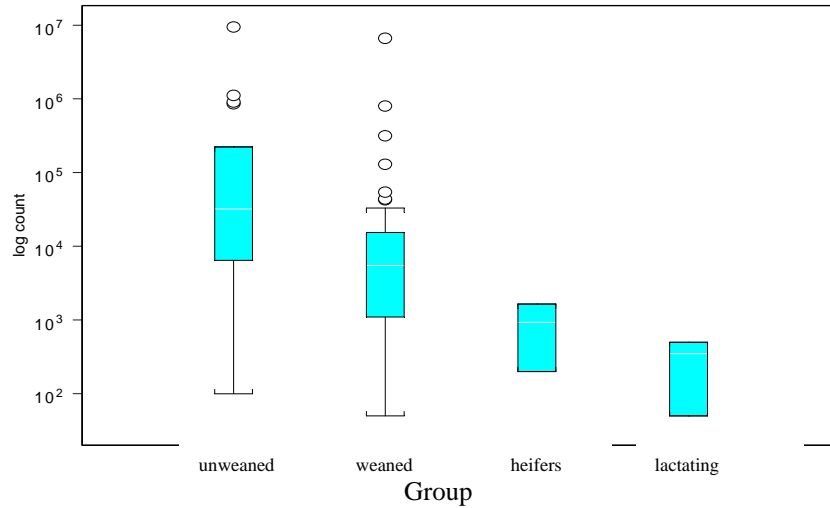


Figure 2. Box plots showing variation in counts of *E.coli* O157 between groups. Boxes contain 50% of the data.

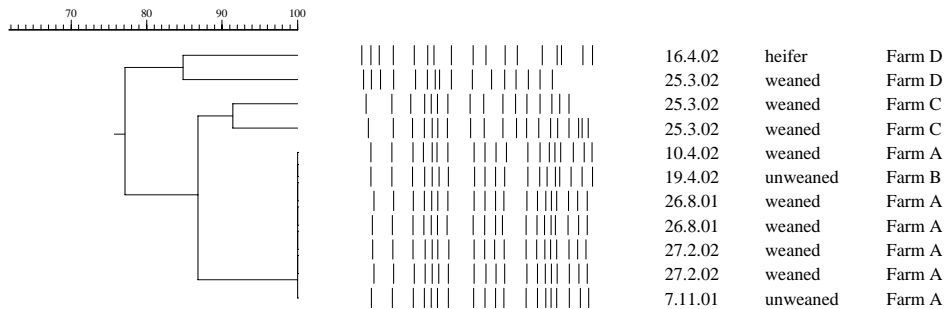


Figure 3. Molecular typing of *E.coli* O157 isolates by farm.

Discussion

The RFLP-PFGE patterns of the isolates suggest persistent strains within farms. Maintenance of these strains within farms could be due to particular management groups. Although the weaned group had a higher prevalence on all farms, the unweaned group consistently shed more bacteria per gram when positive and therefore interventions targeting these two groups may be the most cost effective. Explanations for the high concentrations of *E.coli* O157 seen in unweaned group may be related to the naïve gut microflora providing a niche for *E.coli* O157 colonisation of these pre-ruminant cattle. The indistinguishable strains isolated from neighbouring farms could be due to common environmental pools or be as a result direct contact of animals at farm boundaries.