Short-term temporal variation of *E.coli* O157shedding in naturally infected cattle.

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Abstract

Two cohorts of 14 and 16 calves were sampled intensively for periods of 5 and 14 days to assess the short-term patterns of *E.coli* O157 shedding. *E.coli* O157 in faecal samples were enumerated by spiral plating and isolated by immunomagnetic separation (IMS) to provide both count and presence-absence data. All animals within the two cohorts were found to be shedding *E.coli* O157 during the study with counts varying between 250cfu/g and 1.4x 10^6cfu/g. Some animals persistently shed high numbers of *E.coli* O157 throughout the study. All animals shed strains of *E.coli* O157 that were indistinguishable on RFLP-PFGE.

Introduction

Recent outbreaks of *E.coli* O157 have identified the contamination of both food and environmental sources with cattle faeces as vehicles for human infection. This underlines the importance of reducing shedding in cattle on-farms as a method of reducing risk via multiple exposure pathways. Here we report the findings from two small longitudinal studies of VTEC O157 shedding in naturally infected calves. We explore the patterns of shedding using a multivariate mixed –effects model that combines binary immuno-magnetic separation (IMS) and count data.

Materials and Methods

Two studies were undertaken. In the first study 14 calves aged 6-9 months were sampled approximately every 3hrs for a 5-day period. For the second study 16 weaned calves aged 6-11 months were sampled twice daily for one week and once daily the following week. Samples were taken after animals were observed defecating. Explanatory variables were recorded at the visit, animal and pat level. Explanatory variables at the pat level included colour and consistency scoring. After mixing, 2g faeces were added to 18mls buffered peptone water (Lab M) + vancomycin ((8mg/ml, Sigma Aldrich, UK)). Samples were diluted and plated in duplicate onto Harlequin SMAC BCIG agar (Lab M) supplemented with cefixime (0.05mg/l) and potassium tellurite (2.5mg/l) (Lab M) using a spiral plater (Don Whitely scientific). The remaining sample was enriched at 37^{o}C for 18hrs after which IMS was performed. The bead suspension was inoculated onto CT-SMAC (Lab M) and CHROMagar (M-TechDiagnostics) Presumptive *E.coli* O157 isolates were screened by PCR for presence of *vt* genes and the O157 *rfb* gene. Further isolate characterisation was performed including RFLP-PFGE. An IMS positive result was

assumed to be an accurate indication that the animal was shedding *E.coli* O157 at the time the pat was voided. Denoting by y_{ij} the jth IMS measurement taken from animal i, we model y_{ij} by assuming $y_{ij} = 1$ (a positive result) with probability p_{ij}. The probabilities p_{ij} can vary from animal to animal and depend on covariates such as the colour of the pat sampled and the pat consistency. Formally the model is written as:

$$Y_{ij} \sim Bernouilli(p_{ij})$$

$$\log\left(\frac{p_{ij}}{1-p_{ij}}\right) = \mu_p + A_i + X_{ij}\alpha$$

$$Ai \sim N(0, \sigma^2{}_A)$$

Here the A_i is a random effect corresponding to the animal i, and X_{ij} is a vector of covariates relating to the pat colour and consistency. The plate counts z_{ij} are presumed to be necessarily zero when $y_{ij} = 0$ (the animal is uninfected) and follow a Poisson distribution when $y_{ij} = 1$. The count data are modelled as

$$Z_{ij} \mid y_{ij} = 1 \sim Poisson(\lambda_{ij}, S_{ij})$$

$$\log(\lambda_{ij}) = \mu_\lambda + B_i + X_{ij}\beta$$

$$B_i \sim N(0, \sigma^2{}_B)$$

The S_{ij} is an offset factor depending on the concentration of faeces on the plate and the area of the plate counted. We allow for the possibility that the A_i and β_i are correlated, with corr $(A_i, \beta_i) = \rho$, as we might expect animals which are *E.coli* O157 positive more often to shed higher numbers than animals which are rarely infected. The model was fit in a Bayesian framework and the posterior distributions sampled using Markov chain Monte Carlo methods in the software package Winbugs. We avoid combining the IMS and count data as we wish to examine the relationship between factors affecting infection and factors affecting shedding loads.

Results

In the first study a total of 229 faecal samples were collected at 19 visits over the 5-day period. Of these 113(49.3%) samples were positive on direct enumeration plates and a total of 134 (58.5%) samples were positive by IMS and direct plating. All calves within the pen were found to be shedding *E.coli* O157 on at least one sampling over the 5 days of the study (Fig. 1). There was no obvious diurnal trend. All isolates were *eaeA*, *vt1* and *vt2* positive and had indistinguishable RFLP-PFGE patterns. For the second study a total of 329 samples from 22 visits over the 14-day period. Of these 121(36.7%) samples were positive from direct plating and 151(45.8%) samples were positive after IMS and direct plating. Counts ranged from 250cfu/ml – 4.1 x 10^5 cfu/ml. For the second study the counts ranged from 250cfu/ml – 1.4 x 10^6 cfu/ml. Again all animals within the group shed the pathogen at some point during the fortnight although there appeared to be persistent and transient shedders within the group. Table 1 shows the posterior distributions of model parameters from a sample of size 951 after being thinned by taking only every 10th realisation and discarding the first 500 samples. Convergence of the Markov

chain was assessed by examining the autocorrelation plots of the simulations, examining sample paths, and by running multiple chains. The colour of the faeces sampled was significantly related to both the infection rate and the counts, with darker faeces (colour score 4) being infected more often and containing more *E.coli* 0157. The two animal effects *Ai* and *Bi* are highly correlated, meaning animals which are susceptible to infection shed higher numbers than animals which are mostly non shedders. Faeces of a firmer consistency (score 3) contained more *E.coli* O157 than more fluid faeces (consistency 4 or 5), though solid faeces were not more likely to be infected.

Table1. Posterior distributions for parameters.

Parameter		**mean**	**Sd**	**2.5%**	**97.5%**
Fixed effects					
IMS	μ_p (colour 3 consistency 3)	1.1	0.50	0.15	2.2
	α_1 (colour 4)	0.31	0.043	0.23	0.4
	α_2 (consistency 4)	-0.090	0.049	-0.19	0.0070
	α_3 (consistency 5)	-1.43	0.077	-1.58	-1.27
Count	μ_λ (as above)	1.9	0.35	1.2	2.6
	β_1	0.57	0.3	-0.019	1.2
	β_2	-0.15	0.25	-0.65	0.33
	β_3	0.23	0.37	-0.46	0.95
Randon effects					
Animal	IMS σ^2_A	1.5	0.75	0.66	3.5
Animal	count σ^2_B	2.7	1.6	0.93	7.2
	ρ	0.70	0.16	0.33	0.92

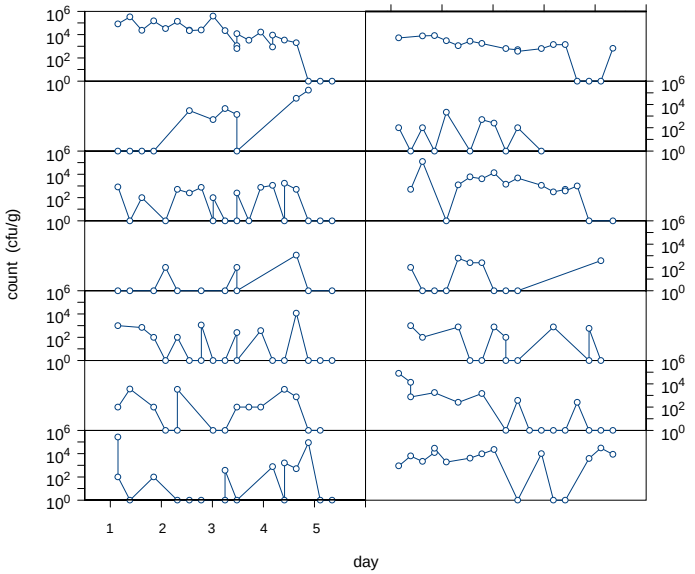

Figure 1. Trellis plots of the individual shedding profiles for the 14 calves in study 1. IMS positive samples with no count are represented as 100cfu/g.

Discussion

Although no diurnal variation was found in shedding, there is large daily and weekly variation in concentrations shed within and between animals. The RFLP-PFGE patterns of the isolates were indistinguishable suggesting that transmission between pen mates may be accounting for the persistency of the strain within the group.