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Factors influencing the shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows

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SUMMARY

A study was designed to investigate management factors that might influence the shedding of verocytotoxin-producing *Escherichia coli* (VTEC) O157 by beef cows in Scotland, where there is a particularly high rate of human infection. Thirty-two herds were visited at least monthly over approximately 1 year for collection of fresh faecal pat samples and information on management factors. The faecal pat samples were tested for VTEC O157 by established culture and immunomagnetic separation methods. Questionnaires were completed at the monthly visits to record management factors. Data were analysed using both univariate and multi-factor (GLMM) analysis. Changes in the number of cows in a group, dogs, wild geese, housing, and the feeding of draff (distillers' grains) were statistically significant as risk factors. The event of calving appeared to reduce the likelihood of shedding. Any effects of weaning or turnout were not statistically significant. It appears that the rate of shedding of VTEC O157 is influenced by several factors but possibly the most important of these are the circumstances of animals being housed, or, when outside, the presence of wild geese.

INTRODUCTION

Escherichia coli O157 is now recognized as an important agent of human disease with world-wide distribution. There are approximately 200 cases of *E. coli* O157 infection in man reported annually in Scotland, where the rate per unit population is consistently four times higher than in England and Wales [1]. Haemolytic uraemic syndrome in the United Kingdom is associated most commonly with verocytotoxin-producing *E. coli* (VTEC) O157. While outbreaks are often food or water related, recent case–control studies have indicated the importance of direct contact with animals as an important risk factor for sporadic cases [2, 3]. It is well known that cattle can be a reservoir of the organism. There is a mass of scientific literature on VTEC O157 in livestock, some of which is highlighted in a recent review [4]. However, little is known about the factors that influence the shedding of VTEC O157 in cattle.

The objective of this study was to investigate management factors that might influence the shedding of VTEC O157 in beef suckler cow herds. VTEC O157

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represent the highest risk to humans, and hence the study monitored the absence or presence of these bacteria in cattle faeces. Although age has an effect on the shedding of VTEC O157 [4], this study focuses on the adult beef suckler cow, despite the fact that various authors have suggested that calves are more likely than adults to be shedding the organism, e.g. in Australia [5], in the United States [6, 7], in the Netherlands [8], and in the United Kingdom [9, 10]. Effort was concentrated on suckler beef cows as these animals remain on farms for considerable periods of time, and therefore long-term data can be collected. In addition, they may serve as an important reservoir of infection, passing VTEC O157 to their offspring that subsequently enter the food chain. Laegreid et al. [11] showed that the widespread infection of beef calves at weaning was the result of infection prior to entry into the feedlots.

Previous studies have shown that the shedding of *E. coli* O157 is typically characterized by short duration, recurrent episodes which may indicate repeated exposure of animals to some source of this agent [12]. Sources that have been hypothesized to be important include persistently shedding individual cattle, other persistent animal reservoirs, and environmental and food-borne sources. VTEC O157 has been isolated from sheep [13], goats [14], wild deer [15], horses [16], dogs [16], geese [1], seagulls [17] and pigs [18]. Animal reservoirs have been reviewed [4].

The organism has been shown to survive in bovine faeces for at least 99 days [19]. Hancock et al. [6] implicated the spreading of cattle slurry on pastureland as a risk factor for the shedding of *E. coli* O157. Swerdlow et al. [20] found sewage contamination of pasture lands or of drinking water supplies to be a source of infection which could result in subsequent spread to crops, animals and man. It has been suggested that diet may influence the shedding of *E. coli* O157 but many of these results are contradictory [4]. Until a consensus is reached, diet cannot be overlooked as a potential risk factor. In addition to the already mentioned hypotheses this study examines the effect of events such as calving, weaning, housing and turnout on the risk of shedding. These events often involve transport, change in feed or other stressors that may be important in the shedding of *E. coli* O157. For example, cattle can pass *E. coli* O157 from one to another [4]. Transmission may be easier between housed animals kept at higher densities and hence in closer proximity.

All the above factors are examined in this study, which seeks associations between them and the shedding of VTEC O157. To the authors' knowledge, no intensive study has been carried out to examine the potential risk factors for shedding in beef-suckler cows. A Canadian study in seven dairy herds [21] showed that shedding in dairy cattle was transient. A longitudinal study of a dairy herd [9], and previous work in Scotland [10], reported seasonal incidence of shedding, but no attempts were made to explain these or assess if trends were statistically significant. A study involving 91 dairy farms in the United States [22] between February and July, showed cattle more likely to shed the organism after 1 May, but no explanation could be given for this phenomenon. More knowledge in this area could lead to the alteration of management practices to try to reduce the shedding of the organism, and therefore contribute to a lessening in the risk to human health.

MATERIALS AND METHODS

Study plan

Between August 1997 and April 2000 32 farms in the north of Scotland were visited. Twelve farms were known to have had a prior history of shedding before the start of the study. The status of the remaining 20 farms was unknown; 16 farms were in Aberdeenshire, and 16 in the Highlands and Islands. Each farm was visited approximately monthly over a 12-month period with the exception of farm A3, which was sampled over a 23-month period. Farm A3 was known to be positive and was the subject of investigation because of a case of human infection with VTEC O157. The farms were not all sampled concurrently. The first four farms in the Highlands and Islands and the first eight in Aberdeenshire were sampled between August 1997 and January 1999, while the remainder were sampled between March 1999 and April 2000 (Fig. 1). Calving was seasonal and generally confined to a maximum 3-month period. In the majority of herds this was February to April, in which case weaning was prior to housing and took place in September. In herds where calving took place in the summer or autumn weaning took place in the spring, before turn-out, i.e. calves were approximately 6 months old at weaning.

Field procedures

On each farm an isolated group of beef-suckler cows were identified for sampling. The size of the groups

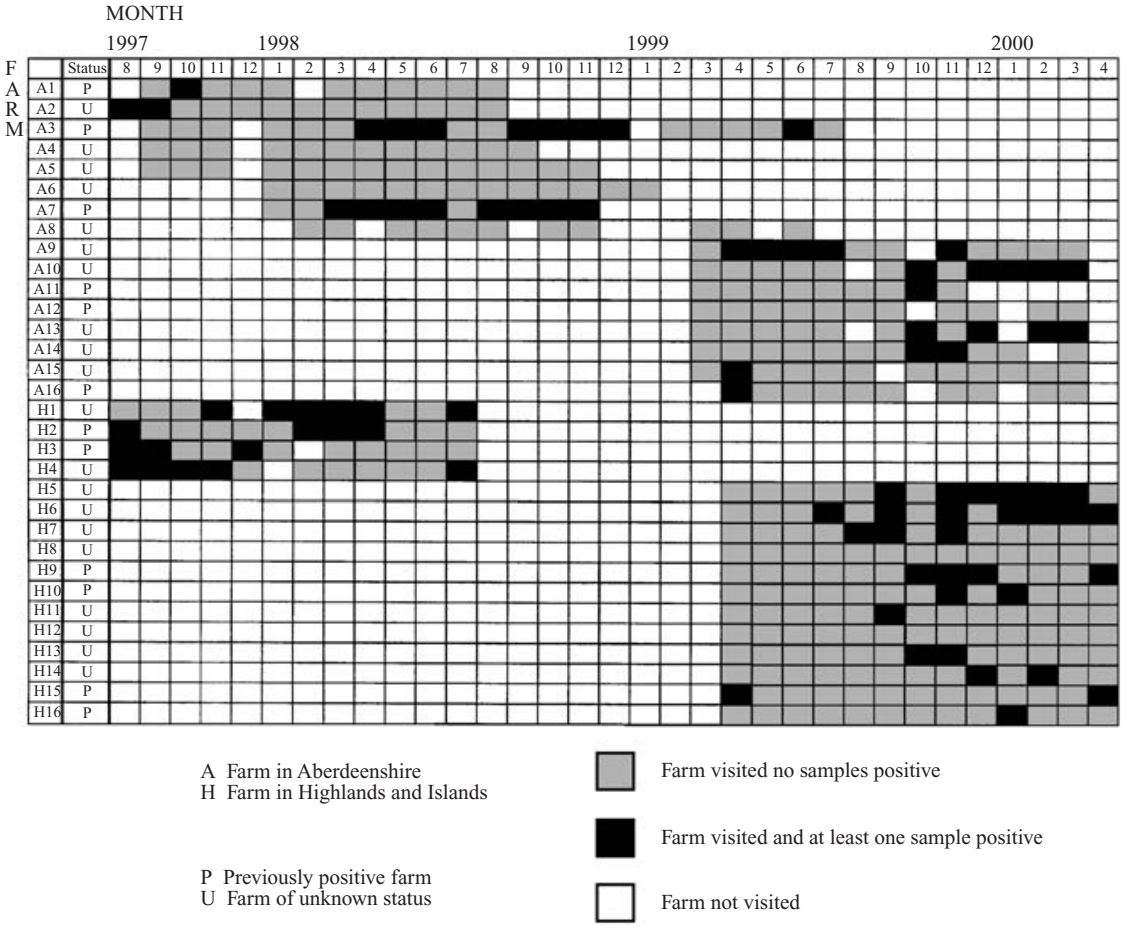

Fig. 1. Pattern of shedding in herds sampled monthly.

ranged from 9 to 100, depending on the farm. This group was followed for the remainder of the study. Once the study began the farms were visited approximately monthly, although some farms were visited more frequently when events such as calving, weaning, housing, or turnout occurred. At each visit a farm management questionnaire was completed and faecal samples were taken and returned to the laboratory for analysis.

Faecal pat sampling

The number of samples collected at each visit was determined using criteria that had been developed for a prevalence study [23]. In summary, sample sizes were generated based on a model of within-herd prevalence, assuming that 2% of herds would contain shedding animals. The shedding patterns on positive farms would be similar to those seen in data from farms previously investigated following human infection, varying around 10%. From this model the number of samples required to give an adequate probability of detecting that a herd contained cattle which were currently shedding was calculated. This power was set at 80%, a biologically acceptable value. For example, in a group of 20 cows, 17 samples were collected, with 30 cows 20 samples, and for 50 cows 23 samples were collected.

Samples were collected from fresh faecal pats into sterile plastic containers. These were tested on the same day except for samples from the two most remote farms, which were posted to the laboratory and tested within a week of sampling.

Farm management questionnaire

There were five operators who collected samples over the course of the study. All received detailed information on the sampling criteria and methodology. Farm personnel were questioned on the following topics: feed, use of fertilizers, water supply, the presence of animals and the timing of events such as calving, housing, weaning and turnout (Table 1).

Table 1. *Factors investigated in the farm management questionnaire*

Category	Specific factors
Food	
Fodder	Hay, pit silage, baled silage, straw, root crops
Concentrates	Home concentrates: barley and others; Bought in concentrates: draff, dark grains, cobs, nuts, others
Others	Minerals
Fertilizers	Organic manure, slurry, human sewage sludge
Water supply	Mains, private, natural
Animals	Domestic (sheep, goats, horses, pigs, poultry, ducks, geese, cats, dogs) and wild (gulls, geese)
Events	Calving, weaning, housing, turnout

Laboratory procedures

Isolation

Three SAC disease surveillance centres at Thurso, Aberdeen and Inverness carried out direct culture on sorbitol MacConkey agar containing cefixime and tellurite (CT-SMAC). In addition 1 g faeces was added to 20 ml buffered peptone water and incubated for 6 h at 37 °C prior to immuno-magnetic separation (IMS) with O157 antibody coated beads followed by culture on to CT-SMAC. CT-SMAC plates were incubated at 37 °C for 18–24 h. Non-sorbitol fermenting colonies were selected and tested for agglutination with *E. coli* O157 latex reagent. The IMS method employed was similar to that described by Chapman [24], but leaving out the antibiotics in the enrichment broth [25]. The justification for this modification of the technique has been described previously [26].

Typing

The reference laboratory carried out confirmatory tests of all isolates as *E. coli* O157, phage typing [27] and examination for the verocytotoxin genes VT1 and VT2 using a multiplex PCR [28]. In addition 332 isolates were tested for the *eae* gene which encodes for enterocyte attachment and effacement [29].

Statistical analysis

Case definition

If VTEC O157 was isolated by either method from any animal in a group on a particular day the group was defined as positive on that occasion for the purpose of analysis.

Farm management questionnaire

Data from all of the visits were recorded. The majority of variables were coded as present or absent on a given visit with the exception of the dynamic events: calving, weaning, bringing in and turnout, which were recorded as having occurred soon before the sampling occasion. Where a sample was taken within the 14 days after the event occurred, the indicator variable was coded as present. Such criteria enabled the variables to be standardized across all farms. A variable for housed was also used in the model to differentiate between groups of animals that were currently housed and those that were grazing. In addition to the above the following quantitative variables were added to the database in preparation for multivariate analysis: the number of faecal samples taken, the number of positive faecal samples and the total herd size. Indicator variables such as 'were there changes in diet?' or 'were there changes in the number of suckler cows in the group since the last sample?' were also created.

Statistical methodology

Most univariate and all multi-factorial methods of analysis were carried out using SAS. Preliminary (univariate) analysis at the farm level was performed on all variables (Table 1) using Odds Ratios (OR). Farms were divided into positive (VTEC O157 was detected in at least one sample) and negative (VTEC O157 was not detected in any sample) and each variable was recorded as being present (recorded on at least one visit) or absent (never recorded on the farm) and summed across farms to create a contingency table. OR were generated from the contingency tables with 90% confidence intervals (CI) while significance was tested using Fisher's Exact test. When examining the dynamic variables, such as calving or turnout, the most meaningful comparison is between the shedding status of farms before and after the occurrence of the event. The numbers of cases where farms switched from one shedding class to the other and where they remained in the same shedding class were recorded for each event (Fig. 2), as were the number of switches or no switches that took place in the absence of the events. The null hypothesis that these pairs of switching rates were equal (i.e. that on balance the event neither encouraged nor discouraged shedding) was tested using Fisher's Exact test.

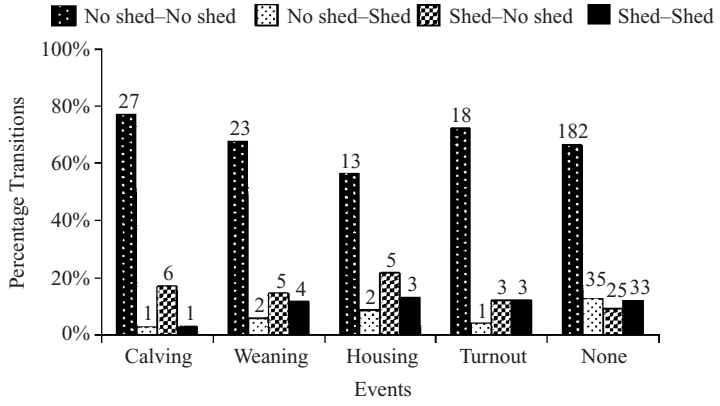

Fig. 2. Prevalence of combinations of shedding patterns of VTEC O157 when evaluated before and after calving, weaning, housing, turnout and null events. No shed = not shedding; Shed = shedding. For example the category No shed–shed summarizes the number of occasions on which no shedding was initially present, but was observed in a set of faeces samples collected within 14 days of the specified event. The figure above each bar represents the number of herd observations counted in a category, as defined with respect to the associated event.

Multi-factorial analysis was performed by fitting a generalized linear mixed model (GLMM) [30] to the number of positive samples from the total number of samples collected on each sampling occasion, using a binomial error distribution and logic link function. The GLMM allows the analysis to explicitly model both within and between farm variation. It also has advantages in handling data from observational studies such as this, where data are often unbalanced, both intentionally (focusing on events of interest) and unintentionally (where values are missing). The GLMM was fitted in SAS using the GLIMMIX macro, with Farm fitted as a random effect. Results were reported with *P*-values $\leqslant 0{\cdot}10$. Ideally, a model for the temporal autocorrelative structure of the within-farm variability would have been incorporated into the analysis. However, such models would not converge, possibly due to the highly unbalanced nature of the dataset. Using an alternative approach to compensate for autocorrelation, an indicator variable called 'Previous Sample' was defined and fitted in the model. 'Previous Sample' defines situations where the previous sample on a given farm included samples which tested positive for VTEC O157. It is reasonable to assume that a farm sample is more likely to be positive if even one of the samples from that farm on the previous visit was positive.

The GLMM was initially fitted with 34 factors and variables and these were reduced using a backward stepwise elimination strategy until all that remained had *P*-values less than or equal to 0·10. Some variables had been removed prior to analysis as they were either not present on any farms or were present on most farms. Such uniform effects would cause the model convergence to fail, and give rise to meaningless parameter estimates. There was evidence of interactions between several of the factors, and these interaction terms were included in the final model. Diagnostics were performed, and plots of residuals and farm-level random effects examined, confirming the goodness-of-fit of the model, while the stability of the model was assessed by determining the response of each variable to the removal of each factor.

RESULTS

Laboratory results

Isolation and typing of VTEC O157

A total of 9256 faeces samples were processed by IMS and from these there were 392 positive for VTEC O157, giving an overall prevalence of 4·2% samples positive. The majority (7818) of samples were also subjected to direct culture but only five were positive by this method. Of 420 *E. coli* isolates collected 417 (99·3%) were confirmed to be *E. coli* O157.

Of the 417 *E. coli* O157 isolates, 25 (6%) were found to be VTEC negative (had no VT genes) and were removed from subsequent analysis. Three hundred and fifty-seven (91%) of the isolates contained the VT2 gene only, 34 (8·7%) contained VT1 and VT2, but only 1 isolate (0·3%) contained VT1 only. All of the VTEC O157 tested were *eae* positive.

Table 2. *Significant associations between factors on positive and negative* E. coli *O157 farms. Values represent counts of the number of farms. Sample size is in brackets*

	Positive	Negative	Odds ratios (90% CI)
Housed	22 (25)	1 (7)	44·0 (5·7–340)*
Home concentrates: barley	18 (27)	1 (7)	15·4 (2·26–105·4)†
Cats	19 (25)	2 (7)	7·9 (1·64–38.3)†
Dogs	20 (25)	3 (7)	5·3 (1·19–23·9)‡

* $P<0{\cdot}001$; † $P<0{\cdot}05$; ‡ $P<0{\cdot}1$.

Field results

Farm-level patterns

Of the 20 farms that were of unknown status at the onset of the study, 14 (70%) tested positive for VTEC O157 in at least 1 sample on at least 1 occasion (Fig. 1). On 7 out of the total 32 farms no VTEC O157 was isolated at any point in the study. Among positive farms, sets of positive samples were isolated on between 1 and 8 sampling occasions. The majority (92%) of positive farms, however, exhibited shedding for less than 5 months during the study (Fig. 1). The longest consecutive period of shedding was a 5-month block seen on farm H5. On most of the farms, however, shedding was detected in blocks of 1 or 2 months which could be separated by non-shedding blocks of anything between 1 and 11 consecutive months.

Univariate analysis showed no effect of farms using fertilizer or spreading manure, the feeding of forage crops or the type of water supply. However, there were significant statistical associations ($P<0{\cdot}001$) between shedding and animals being housed (Table 2). Only 1 of the 7 farms that were negative ever reported animals being housed for a period of longer than 4 days. Besides farms that housed animals, farms that fed home grown barley concentrate ($P<0{\cdot}05$) or had cats ($P<0{\cdot}05$) or dogs ($P<0{\cdot}10$) were also more associated with shedding (Table 2). A small proportion of farms showed a change in the presence or absence of shedding associated with the events of calving, weaning and change in housing (Fig. 2). These effects were only significant for calving, where positive farms which contained calving animals were more likely to convert to negative status at the subsequent observation ($P=0{\cdot}03$); negative farms with calving animals were also less likely to subsequently convert, although this was not formally statistically significant ($P=0{\cdot}07$). Positive farms containing weaning animals were more likely to have retained their status since the previous observation, but this was not formally statistically significant ($P=0{\cdot}07$). The housing of animals was associated with an increased risk of negative farms having converted to positive, while turnout was associated with an increased chance of positive farms having converted to negative, but neither of these effects were close to statistical significance. There was a significant seasonal effect with shedding being high in the autumn and low in the summer ($\chi^2_3=13{\cdot}88$, $P=0{\cdot}003$) (Fig. 3).

Within and between farm patterns

There was variation with respect to time in most explanatory variables at the within-farm level. This variation is likely to be important given the equally variable nature of the shedding of VTEC O157 at the within-farm level. Of the 34 variables that were considered for inclusion in the model, only 4 were significant as main effects (change in number of cows, pigs, dogs, wild geese); a further 4 variables were only significant as interactions with other variables (season, draff (a bought-in concentrate – distillers' grains), housed, and bringing in (Table 3)). Bringing in has to be modelled as an interaction with housed since it is nested within this other factor. The indicator variable 'previous sample' was significant ($P=0{\cdot}0001$) suggesting that there is temporal correlation in the data from individual farms. The inclusion of this variable dampens the significance of the other variables in the model and should allow a more meaningful interpretation of results. Several possible effects at the farm level are believed to be highly confounded. Even after fitting farm as a random effect, the residual deviance suggested that the data was somewhat overdispersed, but this is not unexpected from this type of epidemiological data.

Table 3. *Odds Ratios and 95% CI for estimated effects in the GLMM analysis*

Variable	Estimated effect	S.E.	Odds ratio*	95% CI†	*P*
Main effects					
Change in number of cows	0·82	0·240	2·3	1·42–3·65	0·0007
Pigs	−1·86	0·911	0·2	0·03–0·93	0·04
Dogs	2·15	0·543	8·5	2·95–24·8	0·0002
Wild geese	1·39	0·589	4·02	1·27–12·7	0·0001
Interactions					
Effect of housed and wild geese	3·54	1·05	34·4	4·42–267	0·005‡
Effect of wild geese					
If unhoused	3·29	0·820	27·0	5·42–134	0·004*
If housed	*0·52*	*0·643*	*1·7*	*0·48–5·9*	*1·00*‡
Effect of housed					
If no wild geese	3·02	0·866	20·5	3·76–112	0·003‡
If wild geese present	*0·24*	*1·40*	*1·3*	*0·08–19·6*	*1·00*‡
Given housed animals					
Effect of bringing in	−1·68	0·492	0·2	0·07–0·49	0·0007
Effect of draff	1·23	0·463	3·4	1·38–8·47	0·04‡
Effect of wild geese present					
If summer	3·33	0·910	27·9	4·69–166	0·008‡
If winter	2·78	0·823	16·1	3·21–80·7	0·02‡
Effect of no wild geese present					
Autumn versus spring	1·76	0·407	5·8	2·62–12·9	0·0005‡

* Odds Ratio = exp(estimated effect).
† 95% CI = exp(estimated effect ± 1·96 * S.E.).
‡ Values adjusted using the Bonferroni correction.

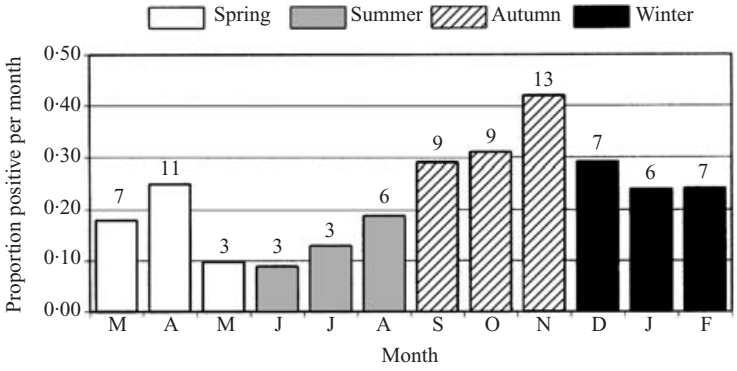

Fig. 3. Seasonal shedding of VTEC O157. The numbers above each bar indicate the numbers of herds with at least one cow shedding in any month.

Further hypothesis testing of parameter estimates was conducted on the significant variables in order to determine the nature of any significant main effects and interactions. Where more than one comparison was made with respect to an effect, a Bonferroni correction was applied. Table 3 lists the OR and 95% CI for the significant effects in the GLMM analysis. The following variables were associated as main effects with a risk of higher shedding ($P<0{\cdot}10$; OR >1): change in the number of suckler cows in the study group, the presence of dogs and the presence of wild geese (Table 3). By contrast, shedding appears to be lower when pigs are present on the farm. The Bringing in by housed interaction was highly significant, representing an apparent protection factor. There were also significant interactions among the following

variables: wild geese, season, housed and draff. Draff was found to interact with housed in a rational manner: only housed animals exhibited higher shedding rates while eating draff ($P=0{\cdot}04$). There was no significant difference in shedding rates among grazing animals related to the feeding of draff. In order to examine the interactions between the other three factors, multiple two-way interactions had to be generated because the model would not converge using three-way interactions because of the sparseness of the data matrix. Housing is a highly seasonal factor. Of the three two-way interactions that were entered into the model, two were significant: season by wild geese and housed by wild geese. Season by housed was not significant.

It is difficult to separate the effects of housing, season and wild geese in these data. If animals are grazing, the presence of wild geese on the farm is a significant risk factor for shedding. Among housed animals, the effect of wild geese is minimal. On farms, which have no wild geese present, housing is a clear risk factor, although on farms with geese present, housing has no apparent extra effect. In both summer and winter, there was statistically significant evidence that the presence of wild geese increased the risk of shedding, although it should be noted that only two farms reported wild geese as being present during the summer. On farms without geese, it was possible to establish that shedding levels were significantly higher in autumn than in spring, once allowance had been made for housed and other significant factors. This was the only seasonal difference that was not explained by some other seasonally variable factor.

DISCUSSION

The herds for this study were not randomly selected; hence it is invalid to make inferences about prevalence levels. However, the status of 20 of the 32 farms sampled was unknown at the beginning of the study, and these can be used to obtain an estimate of the farm prevalence of *E. coli* O157. Of the 20, 14 (70%) were positive at some stage. While a majority of farms were positive on at least one occasion, positive samples were detected only in a minority of visits (22%) (Fig. 1). This figure closely matches the farm level prevalence (23%) found in a concurrent study of beef finishing cattle [23]. This indicates a highly significant risk for persons coming into contact with beef cows or their faeces. Case control studies [2, 3] have indeed suggested that direct contact with cattle or cattle faeces are important risk factors, at least for sporadic cases of *E. coli* O157 in man.

The observed shedding of *E. coli* O157 in this study was transient in nature. Similar observations have been made in other studies [12, 21]. In an experimental study, inoculated calves shed the organism intermittently up to 58 days and cows up to 44 days, but on the majority of sampling days the organism was not detected [31]. Besser et al. [12] found considerable variability in the excretion of *E. coli* O157 by cattle, noting that negative herds can change status suddenly and dramatically.

The vast majority of *E. coli* O157 isolated were verocytotoxin-producing and all those tested had *eae* genes. It has to be assumed, until proven otherwise, that all these isolates are potential human pathogens though there may be a subset of organisms found in cattle that are less likely to be human pathogens [32]. Reports from the United States suggest a different proportional balance of VT types and combinations [33].

One of the key objectives in this study was to test for association between management events and shedding of VTEC O157. However, when considering the power of this study to detect statistical associations for such shedding, it must be remembered that, although this was a larger and more complex study than any previously reported, it generated a relatively small data set at the between-farm level. The results obtained must be interpreted with caution and should be interpreted as indicating possible risk factors for the shedding of VTEC O157 and used to develop further epidemiological hypotheses that can be tested in future studies. Conversely, smaller statistical effects may have been missed by this study.

No association was confirmed between shedding and housing or turnout in the univariate analysis, although it should be noted that housing was associated with relatively more farms becoming positive and turnout with relatively more farms becoming negative. The multi-factor model for shedding found strong effects due to housed and bringing in. Housed was, in general, associated with an increased shedding rate, but relative to this higher baseline, bringing in was associated with a lower shedding rate. This apparent contradiction may arise from other factors which come into play when animals are inside, e.g. close confinement (high population density) or possibly contaminated feed and water supplies. Rahn et al. [21] found feed managers and water bowls had the highest rates of positivity for VTEC O157, suggesting

they may play a role in animal-to-animal transmission. If such factors take some time to affect newly housed animals, those animals recently housed would indeed show a lower level of shedding, making the act of bringing in look like a protective factor. This would merely be an artefact of the inevitable nesting of 'recently housed' within housed. This does not preclude other possibilities, such as the change in ration at the time of housing being protective. A study in Switzerland of 67 cow-calf units showed increased shedding of VTEC in housed calves, but the calves were all older when at pasture than they were when housed [34], confounding age and housing effects. In a study of 36 dairy herds in the United States, no association with housing was detected [35]. It is possible that variation in sanitary levels may generate the variation reported in these studies.

This study found some association between calving and shedding rates (rates dropping after calving was over) and weak evidence of the maintenance of shedding being associated with weaning. This evidence should be treated with some caution since similar evidence was not found in the multi-factor analysis. Faecal shedding of *Salmonella*, spp. is frequently associated with calving and the reason that there is not such a strong association with *E. coli* O157 may be related to a lack of invasion or colonization in cattle. Given the transient nature of shedding it is possible that our study missed any association between some events and shedding. Sampling at more frequent intervals before and after such events would be necessary to provide sufficient coverage to establish these relationships. It should be noted that the power of the study to detect any association was limited, given the relatively small number of calving and weaning events that were recorded.

Testing for associations between the shedding of VTEC O157 and the presence of other animals revealed that both dogs and pigs had significant effects. Cats had been significant in the univariate tests, but this could merely indicate that farms that kept cats all tended to exhibit some other risk factor at the farm level. The presence of dogs on the farm was significantly associated with increased shedding with dogs present on 80% of positive farms but only 43% of negative farms. It might be important to establish whether dogs can act as sources of this agent for cattle. Circumstantial evidence exists where dogs may have carried VTEC O157 from cattle to humans or *vice versa* [36] and another unpublished incident observed by the same author. There is a reported case where an indistinguishable VTEC O157 was isolated from a child and a dog but again it is not certain which was infected first [37].

Unlike dogs, pigs were not a risk factor for the shedding of VTEC O157. However, the presence of pigs appeared to be protective. Although Heuvelink et al. [18] isolated VTEC O157 from Dutch slaughter pigs, others [38] found in a small local survey that pigs were not a major reservoir of infection. *E. coli* O157 isolates from pigs are frequently not verocytotoxin producing [39]. Unfortunately this study contained very few pig farms with which to explore the nature of this association. It is likely that the presence of pigs *per se* is not important, rather that pig farms may tend to implement management practices that favour reduced shedding. This is corroborated by the fact that there was no evidence of the presence of pigs being protective in the univariate analysis, i.e. that the pig factor only becomes statistically significant in the analysis in conjunction with other epidemiological factors. More information would have to be gathered under controlled conditions before the full epidemiology of the shedding of VTEC O157 in cattle and other domestic animals can be properly identified.

Another risk factor was found among the cattle themselves. As this was a field study conducted on working farms it was not always possible to maintain the integrity of the sampling groups. Changes in the number of cattle in the study group were recorded at each visit. These changes may influence levels of VTEC O157 shedding. Increases in numbers may lead to increased shedding when the cows that are introduced are actively shedding O157. A Canadian study in dairy cattle showed that open herds are more likely to shed VTEC O157 [40] and a similar conclusion has been drawn from a concurrent Scottish prevalence study [23].

The GLMM analysis allows the exploration of more complex associations between risk factors. There appears to be a complicated inter-relationship between three of the risk factors analysed: season, housing and the presence of wild geese. Unfortunately this study was not large enough to allow a full analysis of the interaction between all of these variables; however, there are strong indications that these important factors do influence the shedding of VTEC O157 by beef-suckler cows. These findings are consistent with the scenario where housed increases shedding, and the presence of wild geese increases shedding among exposed (grazing) animals, and it is coincidental that the increases in each case happen to be similar.

The presence of wild geese was a significant risk factor when considered in association with the season and housed variables. Previously, VTEC O157 has been isolated from domestic geese [1] and seagulls [17], while large numbers of wild geese were reported as present on certain farms during the study (data not presented). The risk from wild geese, however, is not constant but mediated by management practices. Shedding is significantly higher when geese are present but only among grazing animals. On most farms the cattle graze in the spring and summer, while the majority of animals are brought in the autumn and winter months. Hence, it is not surprising to see the apparent effect of wild geese varying by season, though it is surprising that the strongest (and statistically significant) effects are seen in summer and winter. The summer results should be treated with some caution, since they are driven by the experience of only two farms that reported wild geese in summer with concurrent high shedding levels. The winter results require careful interpretation, since the proportion of housed animals in the winter with geese group is much greater than that in the winter with no geese group. Cattle eating forage made from pastures on which there had been geese might explain the winter results.

Housed proves to be a significant risk factor in the GLMM analysis, both as a main effect and in interaction with bringing in and draff (distillers' grains). Housed animals fed draff were more likely to shed VTEC O157. Draff was never fed on any farm that proved negative for VTEC O157. There is evidence in the literature that some feed can support the growth of the organism [41]; this study would suggest that the feeding of draff should be studied as a possible contributory factor in the shedding of O157.

VTEC O157 cases in man tend to be more common in the summer months [42]. The literature generally describes a corresponding peak of shedding by cattle in the summer, e.g. in the United States [7] or in the Netherlands [8]. However, if the published data are examined carefully the UK shedding peak is in the spring or late summer/autumn [9, 10, 38]. It is the interaction between seasonally variable factors (wild geese, bringing in, housed and BICD feeding) that is likely to be responsible for the different seasonal patterns observed in the univariate and multi-factor analyses.

In this study the seasonal pattern from univariate analysis (in decreasing order of shedding) is autumn > winter > spring > summer. However, after the GLMM has allowed for the effects of other significant explanatory factors, the resulting seasonal pattern (in decreasing order of shedding) is summer > autumn > winter > spring, with all but one of these seasonal differences not being statistically significant. The initial pattern is likely the result of a three-way interaction between wild geese, season and housing. The univariate analysis indicated that autumn and winter were the seasons with the highest shedding. This is the time when the cows tend to be housed and hence are affected by this risk factor for the shedding of VTEC O157. In the multi-factor analysis summer was associated with the highest unexplained shedding, but this effect is very variable and not significantly higher than the other seasons. Focusing only on farms with no wild geese, there is some evidence that the shedding in autumn is significantly higher than that seen in spring (Table 3). This, of course, is in line with earlier reports. This is a complex situation which needs more exploration. In general, purely seasonal patterns should be viewed with caution in the future, with attention rather being focused on other, possibly more informative, management factors.

It appears that the rate of shedding of VTEC O157 is influenced by several factors, possibly the most important of these being the state of being housed and, when outside, the presence of wild geese. A more detailed understanding of the biological basis for the observed variability in shedding will be critical to make use of such information in the development of on-farm control measures.

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