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Prevalence of verotoxin-producing *Escherichia coli* (VTEC) O157 in Swedish dairy herds

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SUMMARY

A prevalence study of verotoxin-producing *Escherichia coli* O157 (VTEC O157) was performed in 371 randomly selected dairy herds distributed throughout Sweden. Faecal and manure samples were collected and analysed by immunomagnetic separation and culturing. Data were recorded for each herd regarding herd size, age of sampled animals and whether, in addition to cattle, the farm kept other animals. VTEC O157 was isolated from 33 (8·9%) of the 371 investigated herds. The prevalence was higher (23·3%) in Halland county than in the rest of Sweden ($P>0·01$). Halland was also the county in Sweden that during the study period had the highest incidence of human VTEC O157 cases. VTEC O157 was not detected on any farm in northern Sweden. Identified risk factors, in the multivariate analyses, for herds being VTEC O157 positive were herd size, geographical localization, presence of pigs on the farm and median age of sampled animals.

INTRODUCTION

During the past two decades verotoxin-producing *Escherichia coli* O157 (VTEC O157) has become a serious zoonotic pathogen worldwide. Of approximately 100 different serotypes of VTEC that have been associated with human VTEC cases [1] VTEC O157 H:7/H$^-$ appears to be the most important [2–5]. VTEC O157 is an important cause of human haemorrhagic colitis in all parts of the world. The infection can resemble non-infectious inflammatory intestinal disease. It is usually self-limiting, but can, in up to 10% of cases, elicit acute renal failure and blood clotting disturbances such as haemolytic uraemic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) [5]. These complications are more common in younger and older humans, causing a high case-fatality rate. Sporadic cases as well as outbreaks of disease caused by human pathogenic VTEC commonly occur throughout the world [6]. VTEC O157 has been isolated from several different species such as cattle, sheep, pigs, horses, rabbits and seagulls [7–10]. However, ruminants appear to be the major reservoir for this bacterium. Ruminants are considered to harbour VTEC O157 without expressing clinical signs of disease although after infection under experimental conditions newborn calves have developed clinical signs [11, 12].

In abattoir prevalence studies on cattle performed in Norway, The Netherlands, Canada and South Yorkshire, UK, figures for VTEC O157 prevalence varied between 0·19 and 15·7% [7, 13–16]. Studies at farm level in Norway, Denmark, England and Wales gave estimated prevalence figures between 1·0 and 38·7% [17–19]. Moreover, a study published by Hancock et al. [20] demonstrated that VTEC O157

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was present in 75% of 36 US dairy herds when repeatedly sampled over a lengthy period of time. There seems to be a seasonal variation in the prevalence of VTEC O157 in cattle with the highest number of positive animals during summer and early autumn [21, 22]. In Great Britain and Finland, regional variations in prevalence among cattle within each country have been reported [23, 24]. Several studies have demonstrated that young cattle shed the bacterium more frequently than older cattle. Especially calves from weaning until 12 months of age are suggested to be a major risk group for shedding VTEC O157 [18, 19, 25].

During the period 1996 to 1999, 286 domestic human cases of VTEC O157 were notified in Sweden, representing an annual domestic incidence of 0·5–1·2 cases per 100 000 inhabitants [4]. The sources of human infections are rarely ascertained, but during 1996–2003 a total of 21 Swedish cattle farms have been identified as VTEC O157 positive in epidemiological investigations when tracing human EHEC cases. Of the farms in Sweden where VTEC O157 has been found, several have kept animals other than cattle [26].

The aim of this study was to estimate the prevalence of VTEC O157 on Swedish dairy farms and to ascertain if there are differences in farm prevalences between different regions in Sweden. Another purpose was to examine the association between VTEC O157 status and farm size, age of sampled animals and presence of animals other than cattle on the farms.

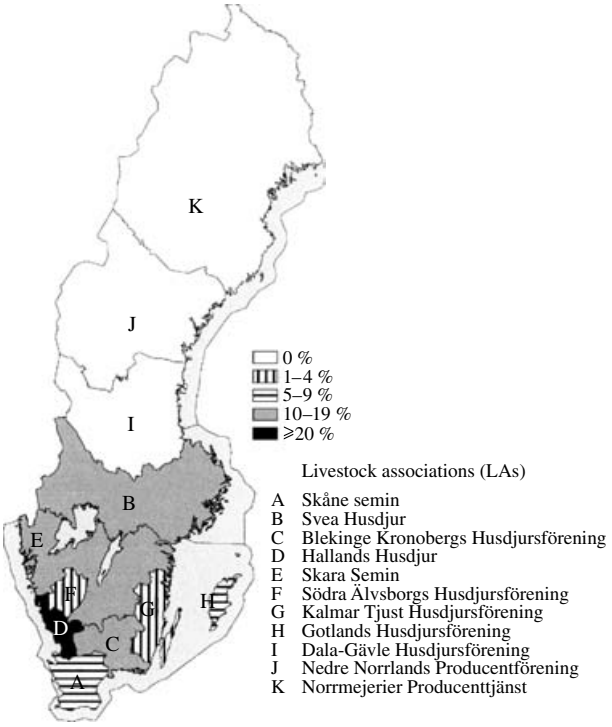

Fig. 1. Geographical distribution of the 11 livestock associations (LAs) and prevalence of VTEC O157 on sampled farms in the different LAs. Results from a prevalence study of VTEC O157 in 371 Swedish dairy herds performed during 1998–2000.

MATERIALS AND METHODS

Design of the study

In Sweden the national milk recording scheme is managed by the Swedish Dairy Association (SDA). Milk recording is undertaken by 11 membership organizations called livestock associations (LAs), geographically distributed as shown in Figure 1. Affiliation to the milk recording system in Sweden is high; during 1998, 10 362 dairy farms (73% of all registered dairy farms) were milk-recorded farms [27].

This study was conducted during three periods: autumn (September–November) 1998, spring (April–June) 1999 and spring (March–June) 2000. For each of these periods farms were selected at random using the SDA's database for milk-recorded farms. The two inclusion criteria were that the farms should be milk-recorded (which usually implies membership of the Swedish Dairy Farm Association) and keep at least 20 young animals on the farm (age 2–12 months). The number of herds to be sampled by the different LAs was proportional to the number of cows in the LAs, but at least five herds from each LA were sampled (Table 1). For the larger LAs the number was rounded off to the nearest multiple of five herds. For each sampling period the 11 LAs were given a list of randomly selected farms, twice the number of farms to be sampled, together with instructions as to how many farms they should sample. If a farm had been sampled before or did not wish to participate, the LA's veterinarian should use the next farm on the sampling list.

Faecal sampling and data collection

During 1998–2000, LA staff visited 371 farms where a total of 7397 individual faecal samples were collected. The numbers of dairy cows (herd size) on the 371 selected farms ranged from 20 to 310 (median 62 cows) (Fig. 2). Visits were performed during the periods: autumn 1998 (124 farms); spring 1999 (125 farms) and spring 2000 (122 farms). The livestock

Table 1. *Distribution of the VTEC O157-positive dairy farms detected amongst the 11 livestock associations* (*LAs*). *Results are presented separately for the three sampling periods, autumn 1998, spring 1999 and spring 2000* (*For geographical distribution of the different LAs in Sweden see Fig. 1.*)

	Proportions of VTEC O157-positive farms of all farms sampled			
LA	Autumn 1998	Spring 1999	Spring 2000	Total (%)
A	1/16	1/15	1/15	3/46 (6·5)
B	2/18	5/19	1/20	8/57 (14·0)
C	2/10	1/10	0/10	3/30 (10·0)
D	1/10	4/10*	2/10	7/30 (23·3)
E	1/31	4/32	4/27	9/90 (10·0)
F	0/10	1/10	0/10	1/30 (3·3)
G	0/10	1/9	0/10	1/29 (3·4)
H	0/5	0/5	1/5	1/15 (6·7)
I	0/5	0/5	0/5	0/15 (0)
J	0/5	0/5	0/5	0/15 (0)
K	0/4	0/5	0/5	0/14 (0)
Total	7/124 (5·7%)	17/125 (13·6%)	9/122 (7·4%)	33/371 (8·9)

* In one herd, VTEC O157 was detected in a manure sample even though it was not detected in any of the four pooled individual faecal samples.

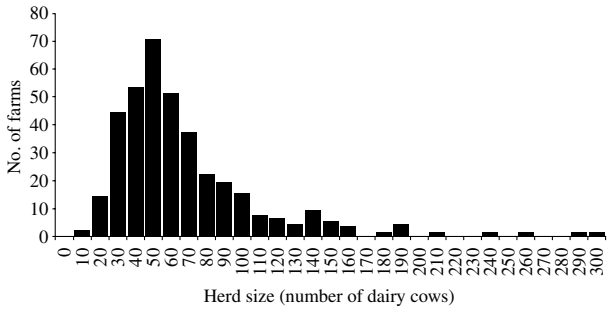

Fig. 2. Distribution of herd size on the 371 dairy farms investigated.

staff were instructed to start collecting faecal samples from recently weaned calves and then continuously move upwards in age categories, sampling animals in order of advancing age, until 20 animals in the age category <12 months had been sampled. From each animal ~ 30 g faeces was collected from the rectum, wearing disposable rectal gloves, and placed in plastic pots. The samples were recorded on an age sampling list in order of increasing age and the age of each sampled animal was recorded. During the sampling period in spring 1999, in addition to the individual faecal samples collected, a manure sample, draining the young stock unit, was collected from all the 125 sampled farms.

Information was recorded at the farm visits regarding the number of dairy cows (herd size) and the presence of any sheep, pigs, goats, horses or poultry and the numbers thereof. For six farms the number of cows (herd size) was not recorded, in these cases this information was retrieved from the SDA's database.

Bacterial analyses of faecal samples

The samples were sent to the National Veterinary Institute (SVA) using the normal postal service. No cooling device was utilized. Bacterial analyses were initiated the day after sampling. The 20 individually collected samples from each farm were upon analyses pooled together 5 and 5 according to the age-related sampling lists (5 g faeces from each sampled animal) resulting in four pooled samples of 25 g faeces. As a consequence of this procedure, samples from calves of approximately the same age were pooled. From the three farms where fewer than 20 samples were received, pooled samples (25 g faeces) were created from fewer than five individual faecal samples. For the 125 manure samples collected during spring 1999, 25 g manure was used in the analysis.

Faecal and manure samples were pre-enriched in 225 ml buffered peptone water for 6–8 h at 37 °C. After pre-enrichment the samples were analysed by immunomagnetic separation (IMS) with paramagnetic beads (Dynabeads anti-*E. coli* O157, Dynal, Oslo, Norway). The IMS step was performed either directly after 6–8 h incubation or after storing the

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incubated pre-enrichment broth at 6–8 °C overnight (16–20 h). Following IMS, the beads were spread out on sorbitol McConkey agar (Oxoid, Basingstoke, UK) supplemented with 0·05 mg/l cefixime and 2·5 mg/l potassium tellurite (CT SMAC). After incubation at 37 °C for 18–24 h, up to five suspected colonies from each agar plate were confirmed by agglutination with a latex kit (Oxoid DR 622). If agglutination was observed, further confirmation was performed by biochemical typing with API 20E (bioMérieux, Lyon, France). Presence of genes encoding for verotoxin 1 (vtx_1), verotoxin 2 (vtx_2), intimin (*eaeA*) and EHEC-enterohaemolysin (EHEC-*hlyA*) and H7 (*flicC*) was determined by PCR technique [28, 29].

Statistical analysis of data

Altogether 371 selected Swedish dairy farms were visited once during 1998–2000 and a total of 7397 individual faecal samples from cattle were collected and analysed as 1481 pooled faecal samples. During 1999, in addition to the faecal samples, one manure sample was collected from each of the 125 herds visited. The agreement between the results from manure and faecal samples was analysed with the kappa statistic and McNemar's χ^2.

The difference in prevalence between LAs was analysed by the likelihood ratio χ^2 test. When examining whether LA D (Hallands Husdjur) had a higher prevalence than the other LAs, Fisher's exact test was applied. The distribution of the positive and negative pooled faecal samples was tested against the hypothesis of a binomial distribution by Fisher's exact test (Table 2).

The analyses of risk factors for positive VTEC O157 findings were carried out using the dairy herd as the epidemiological unit. In the analyses median age was used since median was a more robust measure than the mean (Fig. 3). A generalized linear model (GLIM) with a binomial distribution and a logistic link function was specified (Proc GENMOD, SAS Institute Inc., Cary, NC, USA). Correlation within LA was considered by specifying a compound symmetric correlation matrix in order to obtain the sandwich estimates. These are more robust than the model-based estimates, albeit less efficient, if the assumptions for the models hold good.

The variable 'herd size' could not be assumed to be linear or proportional to the number of cows, as the risk of a herd being VTEC O157 positive would increase by a smaller increment for an increase from 200 to 300 cows than with an increase from 1 to 100. Rather, the incremental risk that a herd might harbour VTEC O157 ought to diminish for each cow added, and the overall risk would asymptotically approximate 1 for an infinite number of cows. Consequently, specification of the variable herd size in the model ought to have these properties. The variable herd size was specified as:

$$1 - \exp[-(\text{number of animals} * \text{prevalence})] \quad (1)$$

(see Fig. 4). This gives the probability of a farm being VTEC O157 positive as a function of the number of cows on the farm and the individual cow prevalence of VTEC O157 found in Sweden. The latter was

Table 2. *Proportion of VTEC O157-positive pooled faecal samples per farm, presented parallel with the predicted distributions according to the hypothesis of binomial distribution* (*Results are from a prevalence study of VTEC O157 in 371 Swedish dairy herds performed during spring 1998 to spring 2000.*)

Proportion of pooled faecal samples positive for VTEC O157	No. of farms	Predicted distributions of pooled faecal samples based on the hypothesis of binomial distribution*
0/4	339	302
1/4	10	64
2/4	7	5
3/4	9	0
4/4	6	0
Total	371	371

* The distribution of the positive and negative pooled faecal samples was tested against the hypothesis of a binomial distribution by Fisher's exact test. The actual distribution differed from that predicted as such, indicating clustering within herds ($P < 0{\cdot}0001$).

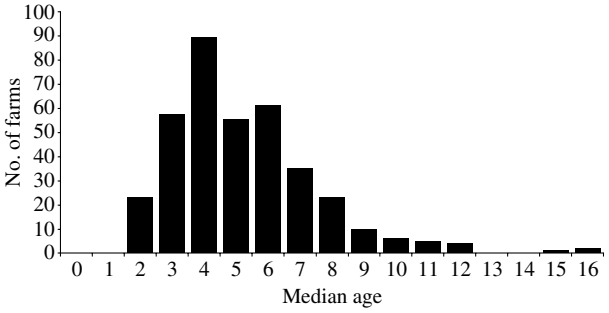

Fig. 3. Distribution of median age of sampled animals in the 371 farms investigated.

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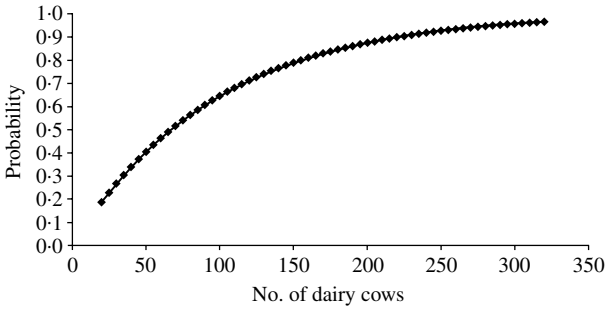

Fig. 4. Relationship between number of dairy cows and probability of a herd being deemed VTEC O157 positive when the variable herd size is presented as 1−exp [−(number of animals * prevalence)] which is the exponential distribution. The total risk asymptotically approaches 1 for an infinite number of cows.

calculated to be 1·034% in the model, or equivalent to that roughly one of every 97 cows were positive, based on the observed prevalence in the pooled samples and the formula:

$$1-\left[\frac{\text{no. of negative pooled samples}}{\text{total no. of pooled samples}}\right]^{\frac{1}{\text{no. of samples pooled}}} \quad (2)$$

The impact of age was analysed as if median age were reduced from 10 to 3 months. The odds ratios were calculated by the formula:

$$\text{OR} = \exp(\Delta X * \beta). \quad (3)$$

For inclusion into the final model a P value of less than 0·1 was required in order to obtain a parsimonious and simple model.

RESULTS

Results from analyses of faecal samples

VTEC O157 was detected in 75 (5·1%) of the 1481 pooled samples, the positive samples deriving from 32 (8·6%) of the 371 dairy herds (Tables 1 and 2). When the results from manure samples and the results from the 1481 pooled samples were combined, VTEC O157 was isolated from of 33 (8·9%) of the 371 sampled herds. Sampling was conducted during three periods, the isolation rate varying between periods with the highest rate during spring 1999. The frequency of VTEC O157-positive farms varied between the different LAs (Table 1, Fig. 1). VTEC O157 was not detected on any farm in the northern LAs (I, J, K); the highest isolation frequency (23·3%) was registered for LA D (Hallands Husdjur), located on the Swedish southwest coast. The hypothesis that the LAs had equal herd prevalence could be rejected ($P<0·05$) and LA D had a significant ($P<0·01$) higher prevalence (23·3% positive herds) than the other LAs (7·6% positive herds) (Table 1). The distribution of the number of positive pooled samples within dairy herds was higher than predicted from the binomial distribution as such, indicating in-herd clustering ($P<0·0001$) (Table 2).

Table 3. *Proportion of VTEC O157-positive pooled faecal samples per farm in combination with the results from the analysis of the collected manure sample* (*Results for the 125 Swedish dairy farms sampled during spring 1999.*)

Proportion of pooled faecal samples positive for VTEC O157	No. of farms where the manure sample for VTEC O157 was		Total no. of farms
	Positive	Negative	
0/4	1	108	109
1/4	1	3	4
2/4	2	2	4
3/4	3	2	5
4/4	2	1	3
Total	9	116	125

Results from the analyses of manure samples in spring 1999

During spring 1999, in addition to the individually collected faecal samples, one manure sample was collected from all the 125 farms visited. The analyses of the pooled faecal samples revealed 16 positive farms, whereas VTEC O157 was detected in the manure samples from nine farms (Table 3). In eight of the nine farms both the pooled samples and manure samples yielded VTEC O157-positive results while on one farm the manure sample proved positive even though VTEC O157 was not isolated from any of the pooled samples. There was a modest agreement between the faecal samples and manure samples, the kappa value being 0·6 (95% CI 0·37–0·84). Thus, it appears that the analyses of faecal samples were more sensitive than those of the manure samples when used to detect VTEC O157 on the farm ($P<0·02$).

Results from the PCR analyses

A total of 84 VTEC O157 strains, 75 from the positive pooled faecal samples and nine from the positive manures sample, were analysed by PCR for presence

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Table 4. *Results from the univariate analyses using VTEC O157 status as the outcome variable and farm as the epidemiological unit of concern* (*Results from a prevalence study of VTEC O157 in 371 Swedish dairy herds performed during spring 1998 to spring 2000*.)

Risk factor	OR	95% CI
Farm size increase, from 20 to 100 dairy cows	4·1	1·4–11·9
Median age decrease, from 10 to 3 months	3·1	1·2–7·6
Other animals (sheep, goats, horses, poultry)	1·4	0·7–2·9
Presence of pigs on farm	2·1	1·1–4·0
Live stock association D	3·0	2·0–4·5
Spring *vs*. autumn	1·9	0·9–3·9

OR, Odds ratio; CI, confidence interval.

Table 5. *Results from the multivariate analysis using VTEC O157 status as the outcome variable and farm as the unit of concern* (*Results from a prevalence study of VTEC O157 in 371 Swedish dairy herds performed during spring 1998 to spring 2000*.)

Risk factor	OR	95% CI
Farm size increase, from 20 to 100 dairy cows	3·5	0·9–12·8
Livestock association D	3·1	1·9–5·1
Presence of pigs on farm	1·9	1·1–3·5
Median age decrease from 10 to 3 months	2·1	1·0–4·7

OR, Odds ratio; CI, confidence interval.

of genes encoding for verotoxin 1 (vtx_1), verotoxin 2 (vtx_2) and intimin (*eaeA*) and at least one strain from all the positive herds was tested for enterohaemolysin (*EHEC-hlyA*) and H7 (*fliC*).

All the tested strains proved positive for intimin (*eaeA*) and all the 33 positive herds evidenced VTEC O157 strains possessing *EHEC-hlyA* and *fliC* genes. When more than one strain was isolated in a herd, all the isolated strains from the herd presented identical vtx_1, vxt_2 and *eaeA* patterns. In 14 (42%) of the 33 positive herds the isolates carried the vtx_2 gene only, while in 19 herds (58%) the isolates carried both vtx_1 and vtx_2 genes. No isolated strains carried the vtx_1 gene only.

Descriptive statistics concerning age

Median ages for the 20 animals sampled in the herds ranged between 2·1 months and 16·5 months (Fig. 3). None of the 33 positive herds had a median age above 8·5 months.

Analyses of risk factors

The results of the univariate and multivariate analyses, with VTEC O157 status as the outcome variable of the herd are presented in Tables 4 and 5. In the univariate analyses (Table 4) the different independent variables were analysed separately, whereas in the multivariate analysis (Table 5) the dependent values included were adjusted to each other in a fitted model. Identified variables that were associated with increasing risk of a herd being VTEC O157 positive in the multivariate analysis were: herd size (increase in number of dairy cows from 20 to 100) [odds ratio (OR) 3·5, 95% confidence interval (CI) 0·9–12·8), if the sampled farm was located in LA D (OR 3·1, 95% CI 1·9–5·1) and if pigs were present on the farm (OR 1·9, 95% CI 1·1–3·5). Furthermore, a reduced median age of the sampled animals in the herds, from 10 to 3 months, was also associated with an increased risk of obtaining positive herds (OR 2·1, 95% CI 1·0–4·7).

In the 371 herds studied, pigs were present in 40 (10·8%) and VTEC O157 was detected in six (15%) of these 40 herds.

DISCUSSION

Prevalence estimation

It is difficult to compare the results from different prevalence studies, as different study designs, sampling procedures and microbiological methods have been used. The number of sampled animals, number of sampled farms, repeated or non-repeated sampling, age of sampled animals, pooling or not of individual samples and amount of analysed faeces all differ between studies.

Hancock et al. [11] suggest that the bacterium is virtually ubiquitous on cattle farms in the United States based on the observation that the number of positive farms increases when farms are sampled repeatedly over a period of time [20]. In a study of 27 dairy farms sampled in England and Wales, eight (29·6%) of the farms proved positive for VTEC O157 [19] whereas in a Danish study a herd prevalence of 17% and an individual prevalence of 3·6% was reported [18]. Lower prevalence was found in a Norwegian study where two (1%) out of 197 sampled

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farms proved VTEC O157 positive and the individual prevalence was 0·3% [17]. In the present Swedish study, the prevalence of VTEC O157 found in dairy cattle fell somewhere between the results of the Danish and Norwegian studies.

Herd size

In our statistical analyses there was an association between the risk of obtaining positive samples and increase in herd size. When the number of dairy cows increased from 20 to 100, the calculated OR for being VTEC O157 positive was 3·5 in the multivariate analysis. Moreover, the finding that the number of positive pooled samples in infected dairy herds was higher ($P<0·0001$) than expected from the binomial distribution as such, supported the hypothesis of transmission of VTEC O157 infection within the herd.

As herd size increases there is probably a rise in infectious pressure up to a certain threshold level. When the number of animals reaches a critical mass, the opportunity for circulation of the bacterium among animals increases and there is also a greater risk of presence of long-time shedding animals. These factors probably promote prolonged persistence of the bacterium in an already infected herd.

A rise in infectious pressure may also result from risk factors inherent in differing management routines in herds of differing size. In larger herds, the movements of calves, young heifers and bulls <12 months old, i.e. the age group considered most likely to shed VTEC O157 [18, 19, 25], are probably larger than in smaller herds. Group penning of calves and introduction of cattle from other herds are other risk factors for VTEC infections [18, 30] that are probably also more common in larger herds. Other management routines that may promote VTEC O157 infection in larger herds include animal movements within the herd, hired labour, different housing, feeding, and grazing systems. Larger herds may also be exposed to more frequent direct or indirect contact with other cattle herds, thus increasing the risk of new infections.

Herd size as a risk factor is not a persistent finding in the literature. In a study performed in the United States, Garber et al. [31] did not observe any statistically significant association between herd size and the shedding of VTEC O157 among dairy calves. However, they did note a significant association with VTEC O157 shedding and the herd's number of heifers in the weaning to breeding age group. In a Danish prevalence study on 60 farms, performed by Nielsen et al. [18], no association between VTEC O157 status and herd size could be found, even though an association was recorded when the herd had relatively many bull calves. In a Canadian study performed by Wilson et al., no relationship was found between herd size and number of cattle excreting VTEC [32]. It is possible that these disparities between other studies and the findings in our study can be explained by the specification of herd size as a linear function rather than as a threshold value. It is also possible that the threshold herd size, as specified in our study, was exceeded on most of the farms investigated in the Canadian and US studies (size of the farms included in these studies was not reported).

Geographical distribution

Other studies have revealed geographical variations in the prevalence of VTEC O157 within countries. In a Finnish abattoir study, Lahti et al. [24] could not isolate VTEC O157 from any sampled animals in northern Finland, whereas the province of Southern Finland yielded 3·3% positive samples. Paiba et al. [23] reported that VTEC O157 was more frequently isolated from abattoirs in eastern Great Britain while the isolation rate was lowest from northern Great Britain. In our study, no VTEC O157-positive farms were identified in the LAs situated in northern Sweden (I, J, K) while a significantly higher risk (OR 3·1) was demonstrated for the herds that were members of LA D situated in southwestern Sweden (Halland county).

Human infections with VTEC O157 are compulsorily notifiable in Sweden. Their incidence in Halland county during 1998–2000 ranged between 2·5 and 4·0 cases per 100 000 inhabitants. These figures were some five- to eight-fold higher than the Swedish national average during the same period [33]. High cattle farm density is a suggested risk factor for VTEC infection on cattle farms [3]. The Halland county has indeed a high cattle density, although it is equally high in other LAs that were included in the study, such as LA A (Skåne semin) and LA G (Kalmar Tjust Husdjursförening), where the registered prevalence was lower, i.e. 6·5% and 3·4% respectively. Cattle densities in the northern LAs (I, J, K) are on the other hand low, a factor that together with the colder climate might explain why VTEC O157 was not detected in any of the 44 herds sampled from these LAs.

Impact of age of sampled cattle

None of the 33 positive herds in this study had a median age above 8·5 months. Furthermore, the statistical analyses demonstrated that the risk of obtaining positive herds increased if the median age of all the sampled animals in a herd decreased from 10 to 3 months (OR 2·1 in the multivariate analysis). Thus, the likelihood of detecting positive herds increased when the proportion of sampled cattle with low age increased. These findings agree with the conclusions of other studies, that cattle in the <6 months old age group are more likely to shed VTEC O157 than older cattle [18, 19, 25].

Influence of pigs regarding prevalence

Studies on pigs as carriers of VTEC O157 are sparse, and little is known about the epidemiology in pigs infected with VTEC O157. Booher et al. [34] demonstrated that persistent colonization of VTEC O157 in pigs can occur up to more than 2 months after artificial infection. Prevalence studies have revealed that the bacterium can be isolated from slaughtered pigs [26, 35].

On the 371 farms studied, pigs were present in 40 (10·8%) herds and VTEC O157 was detected in six (15%) of these 40 herds. A statistically significant association was noted between positive VTEC O157 status and the presence of pigs in addition to cattle (OR 1·9). On the other hand, when the presence of other species was tested as a risk factor, no definite association could be attributed to sheep, goats, horses or poultry.

A monitoring study in The Netherlands also identified the variable of keeping pigs in addition to cattle as being a potential risk factor for VTEC O157 status in dairy herds [36]. Contrary to these findings, in a Scottish study the shedding of VTEC O157 by beef suckler cows appeared to be reduced if pigs were kept on the farm [37].

One explanation for the noted relationship in the present study could be that there are special management routines in these mixed cattle and pig herds that may promote VTEC O157 infections. Another explanation could be that pigs might harbour VTEC O157 to a greater extent than earlier studies have revealed. The prevalence might, for instance, have been underestimated if the bacterium prevails in pigs in very low numbers and if the bacterium is more difficult to detect in pig faeces than in cattle faeces. This low-grade infection would, however, be enough to spread the infection from pigs to cattle if they were kept on the same farm. In a US study [38] VTEC O157 was detected in six (2·0%) out of 305 slaughtered pigs. In that study the authors suggested that the true prevalence of VTEC O157 in pigs in other studies might have been underestimated, as faecal samples from pigs ought to be analysed by different methods than those developed for cattle faeces.

Manure samples *vs.* individual faecal samples

The results from the 125 manure samples, collected during 1999, demonstrated that nine (53%) of the 17 identified positive herds would have been detected if only the manure samples alone had been analysed. In addition, in one herd the analyses of the pooled faecal sample proved negative even though the manure sample was positive. These results indicate that collection of a single manure sample may be an option for VTEC O157 screening in cattle herds if economic resources are limited. The lower cost would enable repeat sampling to compensate for the loss of sensitivity. Nevertheless, the results from manure samples must be interpreted with appropriate caution, as newly introduced, low-level infections may evade detection. Moreover, the manure from which the sample is taken may not originate from all cattle on the farm, due to their segregation.

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