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Risk factors for calf mortality in large Swedish dairy herds

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ABSTRACT

The aim of this study was to identify possible risk factors for 1–90 day calf mortality in large Swedish dairy herds. Sixty herds with a herd size of ≥160 cows were visited once between December 2005 and March 2006. Thirty herds were known to have low mortality (LM) and 30 were known high mortality herds (HM). Upon the visit, data about housing and management was collected from interviews with personnel responsible for the calves. The herd status regarding the calves' passive transfer (total protein), levels of α-tocopherol, β-carotene and retinol, and excretion of faecal pathogens (*Cryptosporidium* spp., *Escherichia coli* F5, rota and corona virus) was evaluated based on targeted sampling of high risk calf groups; in each herd, blood and faecal samples were collected from calves 1–7 and 1–14 days old, respectively. Similarly, the herd status regarding clinical respiratory disease in calves and history of respiratory virus exposure was evaluated based on lung auscultations and blood samplings of calves 60–90 days old. The median calf mortality risk (in calves 1–90 days of age) among HM herds was 9% (Range: 6–24%) and among LM herds 1% (Range: 0–2%). LM and HM herds were compared using five logistic regression models, covering potential risk factors within different areas: "Disease susceptibility", "Factors affecting the gastrointestinal tract", "Factors related to transmission of infectious disease", "Hygiene" and "Labour management". The percentage of calves, 1–7 days old, with inadequate serum concentrations of α-tocopherol and β-carotene were significantly higher in HM herds compared to LM herds and also associated with higher odds of being a HM herd (OR = 1.02; $p = 0.023$ and OR = 1.05; $p = 0.0028$, respectively). The variable "Average number of faecal pathogens in the sampled target group" was significantly associated with higher odds of being a HM herd (OR = 4.65; $p = 0.015$), with a higher average in HM herds. The percentage of calves with diarrhoea treated with antibiotics was significantly higher in HM herds and was associated with higher odds of being a HM herd (OR = 1.08; $p = 0.021$). The median age at death of calves in the age interval 1–90 days that died during a one-year period was significantly lower among HM herds (13 days) than in LM herds (24 days) ($p = 0.0013$) The results indicate that gastrointestinal disorders may be an important cause of calf mortality in large Swedish dairy herds. Furthermore, our study provides additional indications that fat soluble vitamins might play an important role for calf health.

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1. Introduction

High calf mortality is an important cause of economic loss in dairy production (Agerholm et al., 1993; Losinger and Heinrichs, 1997; Mee, 2008). Calf mortality may also retard progress in replacing less productive cows or increasing the herd size (Speicher, 1968; Wathes et al., 2008), and might consequently result in a shortage of replacement heifers and a need to buy animals that further increases the replacement costs of the herd.

Diarrhoea and respiratory infections are the most important disease problems in calves, and enteritis and pneumonia the major causes of death (Virtala et al., 1996; Svensson et al., 2006). *Cryptosporidium* spp., bovine rota and corona viruses (BCV) and *Escherichia coli* F5 are the most important pathogens causing neonatal diarrhoea in calves under Scandinavian conditions (Björkman et al., 2003; Gulliksen et al., 2009a). Respiratory disease is most often initiated by viral agents and bovine respiratory syncytial virus (BRSV), BCV and parainfluenza 3 (PIV-3) are among the most important of these (Autio et al., 2007). Hägglund et al. (2006) found BRSV, BCV and PIV-3 to be common infections in replacement calves in south western Sweden.

Several risk factors for calf mortality have been identified such as routine antibiotic treatment of calf diarrhoea (Lance et al., 1992), group housing (Waltner-Toews et al., 1986b; Olsson et al., 1993), and inadequate passive transfer of colostral immunoglobulin (Jenny et al., 1981; Wells et al., 1996). Inadequate vitamin status has been associated with higher calf mortality during the first week after birth (Lotthammer, 1979). Several authors have reported an association between high calf mortality and increasing herd size (Speicher and Hepp, 1973; Hartman et al., 1974; Lance et al., 1992; Nielsen et al., 2002), whereas other studies found no (Jenny et al., 1981) or little correlation (James et al., 1984). Swedish dairy herds are rapidly getting larger. Between 1997 and 1998, the average dairy herd size was 35 cows, but had increased to 55 cows between 2007 and 2008 (Swedish Dairy Association, 2008). In 2009, 5.4% of the herds affiliated to the Swedish official milk recording scheme had a herd size of 150 cows or more (Swedish Dairy Association, 2010).

Previous Swedish studies have reported low mortality risks in calves 0–90 days of age; 2.6% and 3.1% reported by Olsson et al. (1993) and Svensson et al. (2006), respectively. Higher mortalities have been reported in Denmark (Range: 4.2–13.8%; Nielsen et al., 2002), and the United States (5.6% and 9.4% reported by Virtala et al. (1996) and Losinger et al. (1997), respectively). However, data from the Swedish official milk recording scheme indicate that the mortality risk is getting close to 4% in herds with more than 150 cows (Gidekull et al., 2006). With increasing herd sizes and a trend towards increasing calf mortality in large herds, the calf mortality risk may rise considerably in Sweden in the future.

Although many of the studies on calf mortality in dairy herds during the past 25 years have had similar objectives, the variables examined and the techniques used for analysis have been quite different, which makes direct comparisons of the results difficult. Furthermore, much has changed over time making results from older studies less applicable to the present situation. For example, not only have herds become larger, but there has also been a paradigm shift from treatment of clinical illness to disease prevention (LeBlanc et al., 2006), and new techniques, housing systems and management routines have been introduced. It is well established that management practices affect morbidity and mortality in dairy calves (Kehoe et al., 2007). Studies conducted under prevailing conditions are therefore essential to provide farmers with the latest information and advice matching the present situation.

The objective of this study was to identify risk factors associated with mortality of 1–90 day old calves in large (≥160 cows) Swedish dairy herds known to have high calf mortality risk versus herds known to have low calf mortality risk.

2. Materials and methods

2.1. Study design and selection of herds

2.1.1. Definition of case and control herds

The study was designed as a case–control study and the herd was the unit of concern. The dependent variable was type of herd, classified as case (herds with known high calf mortality risk – HM herds) or control (herd with known low calf mortality risk – LM herds). Herds were classified based on a selection procedure (see Section 2.1.2) in which the herds with the highest and the lowest calf mortality risks among those within the sampling frame were asked to participate in the study. To be classified as a HM herd, the herd's average calf mortality risk had to be *minimum* 6% and to be classified as a LM herd, the average calf mortality risk was allowed to be *maximum* 2%. This design, where extremes are contrasted, was chosen to increase the power of the study, i.e. our ability to detect differences between herds with high and low calf mortality. The underlying assumption is that a strong contrast in the case/control criteria will be reflected in a larger difference between the groups with regards to important risk factors.

2.1.2. Case and control herds – selection criteria

Inclusion criteria for the study were affiliation to the Swedish official milk recording scheme (in 2005, approximately 85% of all Swedish dairy cows were affiliated) and an average herd size of ≥140 cows in September 2003 and ≥160 cows in August 2005. For logistical reasons herds located in the northern part of Sweden, north of the river "Dalälven" (i.e. 6% of the herds in this size range), were excluded. In total, 116 dairy herds met the criteria and were classified as HM or LM herds based on their mortality risk in 1–90 day old calves. They were first ranked based on average mortality risk during the past twelve-month period (September 2004–August 2005) and secondly based on the corresponding mortality risk during the past three months. The 40 herds with the lowest calf mortality risk and the 40 herds with the highest calf mortality risk were invited by mail to participate in the study.

Twenty-five LM herds and 26 HM herds expressed willingness to participate in the study by sending in a written

confirmation. Another five LM and four HM herds agreed to participate after they were contacted by phone. The order in which farmers were contacted by phone was determined by a lottery procedure. One HM herd with an ongoing *Salmonella* Typhimurium infection was excluded because it was under restrictions in accordance with Swedish legislation. A replacement herd was selected using the same procedure as described above. Eventually, 60 dairy herds were included in the study, 30 HM and 30 LM herds.

Following completion of the study, herd classifications were retrospectively validated by calculating the average 12-month calf mortality risk between September 2003 and August 2006 to determine whether the original herd classifications into HM and LM categories were consistent over time.

For the selection and classification of herds, as well as for the retrospective validation, twelve-month herd level mortality risks and herd sizes (measured in September each year) were retrieved from the Swedish official milk recording scheme, covering the period from September 2003 to September 2006.

2.2. Data collection

Each of the 60 study herds were visited once, between December 2005 and March 2006, and all visits were made by the same veterinarian (the corresponding author). Upon the visits, information on farm characteristics, housing, and management and treatment routines on the farm were collected during personal interviews that followed a predesigned questionnaire/interview form. In addition, faecal and blood samples were collected to evaluate the herd status for different factors known to affect calf mortality and morbidity, and lung auscultations were performed to evaluate herd status of respiratory disease. These procedures are described in more detail below.

Birth dates and dates of death for individual calves were not collected on-farm; instead they were retrieved from the National central database for bovine animals.

2.2.1. Questionnaire

The questionnaire used comprised 81 questions, mostly open and semi closed. These dealt with hygiene and calving facilities (e.g. cleaning routines for calf pens, how maternity pens were used and cleaned, proportion individual versus group maternity pens, and proportion cows calving in free stalls), factors affecting disease susceptibility (e.g. colostrum management, location of calf pens), factors relating to transmission of infectious respiratory disease (e.g. age range in group pens, possibilities for direct contact between calves, purchase of animals), factors affecting the gastrointestinal tract (e.g. feeding routines, type of calf starter offered, age when calf starter and roughage was introduced, strategies for treatment of disease) and labour management (e.g. time spent supervising calves, number of calf care takers, number of female calf care takers, staff turnover). Vaccinations, except those against ring worm, are rarely used on Swedish dairy farms; therefore we did not collect data about vaccination strategies.

The questionnaire (in Swedish) is available from the corresponding author upon request.

2.2.2. Interviews

Interviews were carried out with the owners or the personnel responsible for the calves and lasted for approximately two hours. All questions were asked by the veterinarian and were formulated exactly as printed in the questionnaire form, but were further explained if the farmer obviously misunderstood the question. The farmers answered freely on all open questions and all answers were written down.

2.2.3. Sample collection

The herd status was determined using a risk-based approach where individuals at highest risk of exhibiting the condition in question were sampled with priority. This is a cost-effective approach widely and increasingly used for surveillance purposes (Stärk et al., 2006; Schwermer et al., 2009). On each farm, blood samples were collected from calves 1–7 and 60–90 days of age, respectively, and faecal samples were collected from calves aged 1–14 days. Also one bulk milk sample was collected from each farm.

Practical experience have shown that serum concentrations of total protein from three to eight healthy calves 1–7 days of age give a good estimate of the herd's status regarding failure of passive transfer (FPT) of immunoglobulins (de Verdier, National Veterinary Institute, Uppsala, Sweden, personal communication). Jugular blood samples were therefore collected in each herd using this approach. If fewer than three calves in the age interval were available in the herd, no samples were collected from that herd. If more than eight calves $\leq$7 days old were present, the eight oldest of the calves were sampled. The samples (251 samples from 48 herds; 133 from HM and 118 from LM herds) were analysed for serum concentration of total protein and also analysed for antibodies to BRSV, BCV and PIV-3, reflecting maternal antibodies against the viral infections. A subsample of 202 randomly selected serum samples (109 from HM and 93 from LM herds) were also analysed for the serum concentration of α-tocopherol, β-carotene and retinol.

In each herd, faecal samples were taken *per rectum* from up to five calves (287 samples from 60 herds; 140 from HM and 147 from LM), aged 1–14 days. This age group was targeted because we wanted to characterize the herds with respect to the presence of infectious agents causing diarrhoea and affecting neonates, with focus on *E. coli* F5, *Cryptosporidium* spp., BCV and rota virus. The objective was not to assess within-herd prevalence of the agents, but rather to assess whether agents were present or not. The underlying assumption was that if there were a significant problem with any of these agents, the prevalence within the group of interest (calves 1–14 days of age) would be at least 50%. We estimated the expected maximum number of calvings over a two-week period to be 30 (based on the largest herd in the study). In herds with five or more calves in the age group, we needed to sample 5 calves to detect the presence of the agents at the design prevalence, with a confidence of 95% and assuming a specificity of 100% and a sensitivity of 90% (Cameron and Baldock, 1998). If there were fewer than five calves present, all available calves in

that age group were sampled. A sensitivity of 0.9 will possibly be an overestimate for some of the four assays used (see below) (and an underestimate for others), but the decision to work with one design prevalence and one overall sensitivity estimate was made on practical grounds. To further increase the likelihood of detecting the presence of the agents, we adopted a risk-based approach and prioritized sampling of diarrhoeic calves. If no diarrhoeic calves were present, the youngest calves in the age group were sampled; once again to maximize the likelihood of detecting the agents, given they were present.

Also, in each herd jugular blood samples were collected from the ten oldest of the calves 60–90 days old. The samples were analysed for antibodies to BRSV, BCV and PIV-3. This age group was considered to be the one most likely to have been exposed to respiratory viruses.

Salmonella and bovine viral diarrhoea virus (BVDV) infections, which internationally are important causes of calf diarrhoea and mortality, are very rare in Sweden. *Salmonella* status was therefore determined only as present or absent by antibody testing of bulk milk collected by the veterinarian at the visit (one bulk milk sample per herd, but only 58 samples, as in two herds the milk had been delivered just before the visit). Data on the herds' BVDV status were available from the National BVDV eradication programme.

2.2.4. Examination for respiratory disease

The herd's status with regards to respiratory disease was evaluated based on presence of antibodies (in serum) to important respiratory viruses (BRSV, BCV and PIV-3) in individual calves as previously discussed (see Section 2.2.3) and on calves with increased respiratory sounds. On each farm, all heifer calves aged 30–90 days and all blood sampled bull calves (aged 60–90 days) were lung auscultated. In total, 1297 calves were lung auscultated; 644 calves in LM herds and 653 calves in HM herds. The findings were graded as normal respiratory sounds (mild "F-sounds"), or as mildly (strong "F-sounds"), moderately (hissing or sawing breathing sounds with or without bruits), or severely increased respiratory sounds ("K-sounds").

2.2.5. Laboratory analyses of samples

All laboratory analyses were performed at the National Veterinary Institute (SVA) in Uppsala, Sweden, except those for α-tocopherol, β-carotene and retinol which were carried out at Research Center Foulum, Faculty of Agricultural Sciences, Aarhus University, Tjele, Denmark. Antibody tests on bulk milk for *Salmonella* Dublin and *Salmonella* Typhimurium were carried out at the National Veterinary Institute in Copenhagen, Denmark. Samples to SVA were sent by mail on the day of collection and were stored at −20 °C until analysis. However, analyses of serum concentration of total protein were made before the serum was frozen.

The jugular blood samples from calves 1–7 days of age were analysed for concentration of total protein in serum using a refractometer. The samples were also analysed for the serum concentration of α-tocopherol, β-carotene and retinol as described by Jensen and Nielsen (1996).

Presence of *E. coli* in faecal samples was evaluated by culture on McConkey and horse blood agar, and F5 typing was performed using an in-house agglutination test. *Cryptosporidium* spp. was detected using a commercial immunofluorescence kit (Cellab Nordia AB, Sweden), whereas BCV and rota virus was diagnosed using a virus antigen enzyme-linked immunosorbent assay (ELISA) (Larsen, Danish Institute for Food and Veterinary Research, Copenhagen, Denmark) and a commercial rota virus group A antigen ELISA (DAKO, Denmark), respectively.

The jugular blood samples from the 60–90 days old calves were analysed for antibodies to BRSV, BCV and PIV-3 using specific ELISA's (Svanovir®, Svanova Biotech, Uppsala, Sweden). The same analyses were also performed on the sera from 1 to 7 days old calves. According to the manufacturer the test specificity for the ELISA's was 100% for BRSV and BCV and 98.8% for PIV-3. The sensitivity was 94.6%, 84.6% and 95.4% for BRSV, BCV and PIV-3, respectively. The samples were not diluted or centrifuged. The optical density (OD) at 450 nm was corrected by subtraction of the negative control antigen OD. The pooled milk samples from three first-lactation heifers (born in the herd) were analysed for presence of antibodies to BRSV and BCV, using the same methods described above.

Bulk milk samples were tested for antibodies to *Salmonella* Dublin, as described by Nielsen and Ersboll (2004), and against *Salmonella* Typhimurium, using an in-house Mix Elisa (O-antigens: 1, 4, 5, 9, 12; Ref: Feld, Danish Institute for Food and Veterinary Research, Copenhagen, Denmark).

2.3. Data analysis

2.3.1. Data manipulation/editing

Results from samples collected at the individual calf level were transformed to herd level measurements: the variables were dichotomized using cut-offs as described below, and the results were expressed as "Percentage calves with..". The variables describing the herd status with respect to failure of passive transfer (FPT) as measured by total protein, inadequate levels of α-tocopherol, β-carotene and retinol and excretion of important faecal pathogens (*Cryptosporidium* spp., *E. coli* F5, rota and corona virus) were all constructed using this approach. For total protein, samples with <55 g/ml were considered as FPT and for samples analysed for BRSV, BCV and PIV-3, an OD ≥ 0.2 was considered a seropositive result. The cut-offs used for a case (i.e. a calf with an inadequate value) concerning α-tocopherol, β-carotene and retinol were <0.75 μg/ml, <0.25 μg/ml and <0.1 μg/ml, respectively. Also, a variable named "Average number of faecal pathogens in the sampled target group" was calculated by taking the total number of faecal pathogens diagnosed divided by the number of faecal samples taken.

The findings from the lung auscultation were dichotomized into the herd-level variable "Proportion of the examined calves that had moderately to severely increased respiratory sounds at lung auscultation".

"Staff turnover per year" was calculated as the number of personnel working with the calves that had ceased employment on the farm during the past year (2005),

Table 1a
Results from univariable analyses of candidate variables (continuous) for five multivariable logistic regression models with regards to their association with type of herd (high calf mortality (HM; $n = 30$) and low calf mortality (LM; $n = 30$)) in 60 Swedish dairy herds, in a study conducted between December 2005 and March 2006.

	High mortality			Low mortality			*p*-Value
	25 pct	Median	75 pct	25 pct	Median	75 pct	
Disease susceptibility							
% calves, 1–7 days of age, with failure of passive transfer[a,d]	33	54	71	20	31	50	0.020
% calves, 1–7 days of age, with inadequate α-tocopherol status[a,e]	40	77.5	100	20	25	60	0.0063
% calves, 1–7 days of age, with inadequate β-carotene status[a,e]	20	29	40	0	0	20	0.0020
% calves, 1–7 days of age, with inadequate retinol status[a,e]	20	20	33	0	0	20	0.019
Factors affecting the gastrointestinal tract							
Age (days) when concentrate was introduced[e]	9	14	14	4	7	10.5	0.0045
% calves with diarrhoea treated with antibiotics[e]	9	18.5	26	9	9	11	0.042
Factors related to transmission of infectious disease							
Number of animals housed in the same building as calves 4–30 days of age[b,e]	50	77	185	70	170	260	0.074
Relative increase (%) of herd size from March 2004 to March 2005[d]	−3	1.4	5	-0.4	4	9	0.16
% calves, 1–7 days of age, with antibodies to BRSV[a,e]	75	90	100	100	100	100	0.078
% calves, 60–90 days of age, with antibodies to BRSV[c,e]	40	70	100	75	80	100	0.16
Hygiene							
Average number of faecal pathogens in the sampled target group[e]	0.4	0.63	1	0.2	0.2	0.6	0.042
% sampled calves with *Cryptosporidium* spp. infection[e]	0	40	60	0	10	50	0.062
Labour management							
Staff turnover[e]	10	22.5	40	0	15	33	0.15

[a] Variables with 13 missing values (9 LM, 4 HM).
[b] Variable with 1 missing value (1 HM).
[c] Variable with 1 missing value (1 LM).
[d] Difference between high- and low-mortality herds tested with Student's *t*-test.
[e] Difference between high- and low-mortality herds tested with Wilcoxon rank sum test.

divided by the total number of personnel working with calves employed during the same period.

The variable "median age at death" was calculated as the median age at death for all calves in the age interval 1–90 days that died in each herd during a one year period (2005-01-01–2005-12-31).

2.3.2. Statistical analysis

Logistic regression was used to investigate associations between type of herd and potential risk factors, with case/control status (1/0) as the dependent variable. All variables ($n = 237$) were first screened in univariable analyses using the χ^2-test, Student's *t*-test or Wilcoxon rank sum test depending on type of variable (continuous/categorical) and its distribution. Variables associated with type of herd at $p \leq 0.20$ ($n = 25$) were qualified for multivariable analysis (Tables 1a and 1b). The hypothesised causal relationship between the outcome and the candidate variables was outlined using causal diagrams to identify potential confounding effects and intervening variables. The variables were thereafter arranged in five groups (due to the large number of variables in relation to the limited number of herds). They were based on areas of risk factors: "Disease susceptibility", "Factors affecting the gastrointestinal tract", "Factors related to transmission of infectious disease", "Hygiene" and "Labour management". Correlations between variables considered for inclusion in each model were assessed. If highly correlated ($r \geq 0.6$), the variable with the highest explanatory power in the univariable analysis (based on pseudo R^2) was retained. The continuous variable "% of calf pens placed centrally in the stable" was not linearly associated with the logit of the outcome, and it was therefore categorized.

The initial multivariable models were reduced using a manual stepwise backward procedure, with $p < 0.05$ as criterion for retention. Variables excluded during the reduction were re-entered one by one after all remaining variables were significant. Herd size was considered as potential confounder in every model and for the model "Labour management" confounding by the variable "Percentage females working with the calves" was also evaluated. The strategy was to keep confounders in the model if the estimates of the coefficients in a model changed >20% with the potential confounder present. However, in the end no variables were included as confounders based on this criterion. Biologically plausible two-way interactions between the potential risk factors were tested in all models. The fit of the models was evaluated with Hosmer–Lemeshow goodness-of-fit test, with the data partitioned into 6 deciles and with a significant result indicating a poor fit of the model. Plots of Pearson's residuals (r), leverage (h), delta beta ($\Delta\beta$), delta deviance (ΔD) and delta chi^2 ($\Delta\chi^2$) versus the predicted values were constructed to identify potential influential observations (Dohoo et al., 2009). Observations with divergent values, i.e. $-3 \leq r \geq 3$, $h > 0.3$, $\Delta\beta > 1.0$, $\Delta D > 4.0$ were considered outliers and their influences on the models were evaluated.

For seven variables ("% calves, 1–7 days of age, with FPT", "% calves, 1–7 days of age, with inadequate α-tocopherol status", "% calves, 1–7 days of age, with inadequate β-carotene status", "% calves, 1–7 days of age, with inadequate retinol status", "% calves, 1–7 days of age, with antibodies to BRSV", "% calves, 60–90 days of age, with antibodies to

Table 1b
Results from univariable analyses (χ^2-test) of candidate variables (categorical) for five multivariable logistic regression models with regards to their association with type of herd (high calf mortality (HM; $n = 30$) and low calf mortality (LM; $n = 30$)) in 60 Swedish dairy herds, in a study conducted between December 2005 and March 2006.

Name of model/Variable		High mortality	Low mortality	*p*-Value
Disease susceptibility				
Colostrum from cows recently introduced to herd is used	Yes	15	22	0.063
	No	15	8	
% of calf pens placed centrally in the stable	≥50%	12	18	0.12
	<50%	18	12	
Factors affecting the gastrointestinal tract				
Age when roughage is introduced	≤7 days	14	21	0.067
	>7 days	16	9	
Type of concentrate used	Commercial (Pelletted calf feed)	16	23	0.058
	Other	14	7	
Factors related to transmission of infectious disease				
Age difference (oldest to youngest calf) in group pens	≤21 days	17	12	0.20
	>21 days	13	18	
Purchase of ≥10 cows during the past year	Yes	16	8	0.035
	No	14	22	
Hygiene				
Use of maternity pen	For calving + diseased cows	15	22	0.063
	Only for calving	15	8	
% calves with more than one feacal pathogen	≥20%	13	7	0.10
	<20%	17	23	
% cows calving in the free stalls	≥10%	15	10	0.19
	<10%	15	20	
% group maternity pens of all calving pens in the herd	≥45%	19	13	0.12
	<45%	11	17	
Labour management				
Proud to show calves to visitors	Yes	13	18	0.20
	No	17	12	
Standard operating procedures for management routines exist	Yes	19	12	0.071
	No	11	18	

BRSV" and "Number of animals housed in the same building as calves 4–30 days of age") there were missing observations. For the two variables last mentioned, only a small number of observations were missing (<5%), but for the other five variables up to 13 herds had missing values. In these herds there was a lack of calves to sample at the visit, most often because bull calves had been sold at four days of age. In multivariable regression analyses, records with missing observations are automatically omitted, which may introduce serious bias (Rubin, 1987). It is therefore important to address this issue. In this study, another specific reason for concern was the limited sample size, where loss of data also would lead to a decreased power of detecting differences between the groups. Therefore, the missing values for these seven variables were imputed. Multiple imputation (MI) by chained equations as suggested by van Buuren and Knook (1999) was used. Five sets of data with imputed missing values were created using all candidate variables for the model in question as predictors. The regression analysis was then run on all datasets and the results were combined as described by Rubin (1987). This approach produces unbiased results if the underlying assumption that values are missing at random, or missing completely at random (MCAR), is met (Harel and Zhou, 2007). Models with variables with imputed values were rerun without the imputed values to evaluate their effect and the risk of bias. All statistical analyses were done using the software Stata (StataCorp. Stata Statistical Software: Release 9.0; College Station, TX, USA).

3. Results

3.1. Descriptive data

The median 12-month calf mortality risk in calves 1–90 days of age among HM herds was 9% (Range: 6–24%) and among LM herds 1% (Range: 0–2%). Most of the selected herds had a stable 12-month calf mortality risk during the period September 2003–August 2006. However, for two LM herds and for three HM herds, the average 12-month calf mortality risk over the three years was >4% and <4%, respectively. Consequently, their classification as LM/HM was not as stable as for the other herds. All models were run without these herds to evaluate the effect of this potential bias, but overall, the results remained the same in all models. The median age at death of all calves (1–90 days of age) that died in each herd during a one year period was 13 days (Range: 4–39 days) in HM herds and 24 days (Range: 6–66 days) in LM herds.

The median herd size in the HM group was 218 cows (Range: 178–512 cows), whereas it was 219 cows (Range: 166–532 cows) in the LM group. All herds housed their lactating cows in a free stall barn. Calves were most commonly housed in single pens during the first two weeks of life (42 herds); thereafter they were mostly kept in groups of different sizes. Two herds housed their calves in single pens until 90 days of age.

Calves were fed whole milk during their first 4 weeks of life in 43% of the herds and milk replacer in 57% of the herds.

Table 2
Descriptive statistics on the presence of faecal pathogens in 287 faecal samples collected from 1 to 14 day old calves in 30 large Swedish dairy herds with high calf mortality risk ($n = 140$) and 30 herds with low mortality risk ($n = 147$) mortality risk in calves 1–90 days of age. The study was conducted between December 2005 and March 2006.

	High mortality			Low mortality		
	25 pct	Median	75 pct	25 pct	Median	75 pct
Number of faecal samples/herd	5	5	5	5	5	5
Proportion (%) of diarrhoetic samples/herd	0	27	60	0	0	20
Proportion (%) of samples positive for *Cryptosporidium* spp.	0	40	60	0	10	50
Proportion (%) of samples positive for rota virus	0	10	40	0	18	20
Proportion (%) of samples positive for corona virus	0	40	60	0	10	50
Proportion (%) of diarrhoetic samples positive for *Cryptosporidium* spp.	0	20	40	0	0	0
Proportion (%) of diarrhoetic samples positive for rota virus.	0	0	0	0	0	0
Proportion (%) of diarrhoetic samples positive for coronavirus.	0	0	0	0	0	0
Age (days) of non-diarrhoetic calves sampled	2	4	7	3	5	8
Age (days) of diarrhoetic calves sampled	6	8	10	5	9	11.5

In 19 herds, the calves were fed milk *ad libitum* whereas 41 herds fed restricted volumes. The milk volumes given ranged from 4 to 14 l (median 7.5 l) per day. Roughage was introduced at a median age of 7 days (Range: 0–30 days), most commonly hay was fed *ad libitum* (68% of the herds). Age when concentrate was introduced was in median 10 days for all herds. Commercial concentrate (pelletted calf feed) was fed in 65% of the herds, while 22% fed total mixed ration, and the rest of the herds (13%) fed the calves crushed grain. The calves were weaned at a median of 70 days (Range: 38–110 days).

The distribution of pathogens in faecal samples collected in HM and LM herds is described in Table 2. *Cryptosporidium* spp., rotavirus, coronavirus and *E. coli* F5 was found in 32.4%, 17.8%, 4.9% and 0.3% of the collected samples ($n = 287$), respectively. Among the 58 (28 LM and 30 HM herds) bulk milk samples analysed, one LM herd was antibody positive for *Salmonella* Dublin and one HM herd was antibody positive for *Salmonella* Typhimurium. Two herds in the LM group were BVDV infected.

3.2. Univariable analyses

In addition to the variables presented in Tables 1a and 1b, "The median age at death" ($p = 0.0013$), "Proportion of the examined calves that had moderately to severely increased respiratory sounds at lung auscultation" ($p = 0.022$), and "Proportion of faecal samples from diarrhoeic calves" ($p = 0.015$) were significantly associated with type of herd in the univariable analyses (data not shown). These variables were not considered as risk factors *per se*, but rather as features related to the case definition. From a causal pathway point of view, they can be considered as intervening variables and they were therefore not included in the multivariable models, where the association between different risk factors and having high/low calf mortality was investigated. In HM herds, calves died younger and a higher proportion of examined calves had increased respiratory sounds and diarrhoea.

3.3. Multivariable analyses

The results of the multivariable analyses are presented in Table 3.

3.3.1. Model "Disease susceptibility"

To give an example of the magnitude of the associations for the continuous variables, we have calculated what impact a change from the first to the third quartile has on the odds of being a HM herd. An increase in the percentage of calves with inadequate α-tocopherol serum concentrations, i.e. from the 25th percentile (20%) to the 75th percentile (100%) increased the odds of being a HM herd 6.3 times (95% confidence interval 1.2–36.2). A corresponding change in the percentage of calves, 1–7 days old, with inadequate serum concentrations of β-carotene from 0% to 33% increased the odds of being a HM herd 5.3 times (95% confidence interval 1.4–19.7). The percentage of calves with FPT was higher in HM herds, but the variable was not retained in the model ($p = 0.097$). The estimates above are based on the analysis of the non-imputed dataset with a reduced number of herds ($n = 47$, 26 HM; 21 LM). The results from the analysis of the imputed dataset were close to identical, both in terms of variables retained in the model and point- and interval estimates.

3.3.2. Model "Factors affecting the gastrointestinal tract"

To introduce concentrate later in calves' life was associated with type of herd. A change from the 25th percentile (introduction at day 7) to the 75th percentile (introduction at day 14) increased the odds of being a HM herd 2.1 times (95% confidence interval 1.1–4.0). A similar change in percentage of calves with diarrhoea treated with antibiotics from 9% calves treated to 26% calves treated increased the odds of being a HM herd 2.0 times (95% confidence interval 1.0–4.0).

3.3.3. Model "Factors related to transmission of infectious disease"

To have more than 21 days in age difference (oldest to youngest calf) in group pens came out as a protective factor in the analysis of the non-imputed dataset (reduced number of herds, $n = 46$, 25 HM; 21 LM), but was non-significant when all herds were included in the model. The number of animals housed in the same building as calves 4–30 days of age also came out as a protective factor as a higher number of animals housed in the same building reduced the odds of being a HM herd. A change from the 25th percentile (55 animals) to the 75th percentile (257 animals) reduced the odds

Table 3
Results from five multivariable logistic regression models showing associations between various risk factors and type of herd (high calf mortality; $n = 30$) and low calf mortality; $n = 30$) in 60 Swedish dairy herds, in a study conducted between December 2005 and March 2006.

Name of model/variable	Based on original dataset (non-imputed values)					Based on imputed data				
	b	SE (*b*)	OR	95% CI	*p*- Value	*b*	SE (*b*)	OR	95% CI	*p*- Value
Disease susceptibility[a,b]										
% calves, 1–7 days of age, with inadequate α-tocopherol status[c]	0.023	0.011	1.02	1.00–1.04	0.023	0.024	0.00083	1.02	1.002–1.05	0.0052
% calves, 1–7 days of age, with inadequate β-carotene status[c]	0.050	0.020	1.05	1.01–1.09	0.0028	0.043	0.016	1.05	1.002–1.09	0.0012
Factors affecting the gastrointestinal tract[d]										
Age (days) when concentrate was introduced	0.10	0.050	1.11	1.01–1.21	0.018	No missing values				
% calves with diarrhoea treated with antibiotics	0.052	0.023	1.08	1.01–1.10	0.021	No missing values				
Factors related to transmission of infectious disease[e,f]										
Age difference (oldest to youngest calf) in group pens 0 ≤ 21 days/1 ≥ 21 days	−2.50	0.96	0.08	0.012–0.54	0.0028	NS				
Number of animals housed in the same building as calves 4 –30 days of age[g]	−0.0099	0.0038	0.99	0.98–0.997	0.0028	−0.0076	0.0028	0.99	0.988–0.999	0.0085
Purchase of ≥10 cows during the past year (1 = Yes/0 = No)	3.12	1.11	22.66	2.59–198.03	0.0005	2.00	0.71	5.87	1.56–22.14	0.0042
% calves, 60–90 days of age, with antibodies to BRSV[h]	−0.034	0.017	0.97	0.94–0.999	0.020	−0.032	0.013	0.97	0.95–0.997	0.016
Hygiene[i]										
Average number of faecal pathogens in the sampled target group	1.53	0.68	4.65	1.23–17.56	0.015	No missing values				
Use of maternity pen (1 = For calving + diseased cows/0 = Only for calving)	−1.41	0.61	0.24	0.74–0.81	0.016	No missing values				
Labour management										
All over model was not significant										

[a] Hosmer–Lemeshow goodness-of-fit test = 0.81 (non-imputed).
[b] No. of herds when analysis is based on the original dataset (non-imputed values) HM: $n = 26$; LM: $n = 21$.
[c] Variables with 13 missing values (9 LM, 4 HM).
[d] Hosmer–Lemeshow goodness-of-fit test = 0.36.
[e] Hosmer–Lemeshow goodness-of-fit test = 0.51 (non-imputed).
[f] No. of herds when analysis is based on the original dataset (non-imputed values) HM: $n = 25$; LM: $n = 21$.
[g] Variable with 1 missing value (1 HM).
[h] Variable with 1 missing value (1 LM).
[i] Hosmer–Lemeshow goodness-of-fit test = 0.88.

of being a HM herd, with an odds ratio of 0.13 (95% confidence interval 0.03–0.60). The estimates of the effect of this variable changed marginally when based on the imputed dataset. The percentage of calves 60–90 days of age with antibodies to BRSV was highest in LM herds. An interquartile change from 60% to 100% reduced the odds of being a HM herd with an odds ratio of 0.26 (95% confidence interval 0.07–0.95). The estimates for this factor also changed only marginally when the analysis was based on the imputed data. Another factor that increased the odds of being a HM herd was the purchase of more than 10 cows during the past year. The analysis of the dataset with missing observations resulted in a questionably high OR and a wide confidence interval for this variable (still with an acceptable fit based on regression diagnostics and the HL-test though), but running the analysis on the imputed dataset remedied this behaviour to some extent, and provided more reasonable estimates.

3.3.4. Model "Hygiene"

An increase in the average number of faecal pathogens in the sampled target group from the 25th percentile (average number of faecal pathogens 0.2) to the 75th percentile (average number of faecal pathogens 0.8) increased the odds of being a HM herd 2.5 times (95% confidence interval 1.1–5.6). The use of maternity pens differed between herd types. In 50% of HM herds and 27% of LM herds, the maternity pens were only used for calvings. The rest of the herds used the maternity pens for both calving and diseased cows and doing so reduced the odds of being a HM herd.

3.3.5. Model "Labour management"

None of the variables considered for this model were significant in the multivariable context.

4. Discussion

4.1. Model "Disease susceptibility"

The finding that inadequate serum concentrations of α-tocopherol and β-carotene were associated with type of herd is in line with previous studies. An increased risk of diarrhoea during the first week after birth in calves born to dams deficient in β-carotene and in calves with low serum

levels of β-carotene has been shown (Lotthammer, 1979; Puls, 1994; Sivula et al., 1996a; Kume and Toharmat, 2001). Furthermore, several studies have reported that the serum levels of α-tocopherol are important for the immune system of the calf, e.g. by stimulating immune cell function (Reddy et al., 1986; Rajaraman et al., 1998; Higuchi and Nagahata, 2000). The immune defense is essential for the resistance to infectious diseases. α-Tocopherol can also act as an antioxidant (Bendich, 1993). Only very small amounts of α-tocopherol and β-carotene are transferred from cow to calf via the placenta (Van Saun et al., 1989; Nonnecke et al., 1999; Zanker et al., 2000). Therefore, it is essential that the calf is supplied with adequate amounts of α-tocopherol and β-carotene via colostrum. If the vitamin status of the dam is low before and at calving the concentration in colostrum will also be low, resulting in less vitamins for the calf. For best effect, the vitamins should be supplemented via colostrum within 6–7 h after birth (Zanker et al., 2000). In our study, a correlation between FPT and inadequate levels of β-carotene or α-tocopherol at herd level was not identified, which indicates that the reason for differences is inadequate levels of immunoglobulin and vitamins in colostrum rather than inadequate feeding of colostrum. Our results imply that evaluating calves' vitamin status can be of clinical value in herds with high mortality problems.

Different serum concentrations have been suggested as cut offs for what should be considered as inadequate levels of α-tocopherol, β-carotene and retinol in the literature (Pehrson and Hakkarainen, 1986; Herdt and Stowe, 1991). We used a lower cut off (i.e. a more strict case definition) to reduce the risk that calves were falsely classified as cases.

FPT did not remain in the model, which was somewhat surprising. Calves with FPT have been demonstrated to have an increased risk of death (Donovan et al., 1986; Rea et al., 1996), although Sivula et al. (1996b) found no differences in mortality between calves with and without adequate levels of immunoglobulin. However, we acknowledge that the relatively small number of herds in our study reduces the power to detect any minor differences.

4.2. Model "Factors affecting the gastrointestinal tract"

Percentage calves with diarrhoea treated with antibiotics was higher in HM herds than in LM herds, in line with the trend reported by Lance et al. (1992). The data was collected at the herd visits and reflects the herds' management strategies rather than being a direct measure of the percentage calves treated. The result might be due to farmers in HM herds being less reluctant to use antibiotics to treat their diarrhoeic calves since they have lost many calves, or to diarrhoeic calves showing more severe clinical signs in HM herds. The latter explanation is supported by our finding of an association between average number of faecal pathogens in the sampled target group and type of herd in the model "Hygiene". The result may also be due to antibiotic treatment causing an imbalance of the gastrointestinal flora, making the calves more susceptible to pathogens (Roy, 1980). Sivula et al. (1996a) reported increased enteritis morbidity in farms where healthy calves were given prophylactic antibiotic injections. In a recent study, Berge et al. (2009) found that calves treated prophylactically with antibiotics had higher incidence of diarrhoea compared to calves that were treated only when showing clinical signs of diarrhoea with fever or depressed attitude. Sawant et al. (2005) reported that the most common indication for antibiotic usage in dairy calves was enteritis and that 70% of 113 Pennsylvanian farms fed medicated milk replacers. In Sweden, prophylactic use of antibiotics is prohibited and the farmers need a prescription by a veterinarian to get antibiotics for treatment of clinical disease. Ortman and Svensson (2004) reported that diarrhoea was the second most common cause for treatment with antimicrobial drugs (next to respiratory disease) and that 86.4% of such treatment was given within 90 days of age. A reflection is that in our study, only one sample out of 287 was positive for *E. coli* F5, i.e. contained a bacterial pathogen against which antibiotics could theoretically be effective. Instead *Cryptosporidium* spp. and rota virus were the most common pathogens, indicating that overall, the beneficial effect of antibiotics should be limited.

To introduce concentrate at a higher age was associated with an increased odds of being a HM herd. Ingestion of concentrate stimulates the differentiation of the ruminal absorptive epithelium of the young calf into its characteristic papillae (Drackley, 2008). A well developed epithelium earlier in life might give calves in LM herds improved conditions for growth and also increase their resistance to pathogens.

4.3. Model "Factors related to transmission of infectious disease"

Opposite of what was expected, a larger age difference between the oldest and youngest calves in group pens fell out as a factor that decreased the odds of being a herd with high calf mortality. This is in contrast to a recent study in Norway, where Gulliksen et al. (2009b) showed an increased risk of respiratory disease, if the age difference between calves kept in the same group pens was more than 56 days, compared with calves of similar age kept together. However, it should be noted that this variable was not significant in the analysis of the MI dataset. As previously mentioned, loss of data can introduce bias, and we would therefore suggest a conservative approach and avoid drawing any further conclusions based on this finding.

According to the results of the univariable analysis, a higher proportion of calves with moderately to severely increased respiratory sounds at lung auscultation increased the odds of being a herd with high calf mortality. Also, Sivula et al. (1996b) suggested stocking density as a way of determining housing as a risk factor for calf pneumonia. It was therefore surprising that a higher number of animals housed in the same building as calves 4–30 days of age decreased the odds of being a HM herd, as the opposite was expected. A similar effect was seen with the percentage calves 60–90 days of age with antibodies to BRSV, which also decreased the odds of being a HM herd. However, maternal antibodies to BRSV can be detected for long periods and the higher percentage of BRSV positive calves in LM herds might be an indirect reflection of the mothers' BRSV

status (Elvander, 1996) and/or an effect of better colostrum routines. Consequently, one interpretation could be that calves in LM herds are better protected in case of a challenge with BRSV, and develop less severe clinical response with fewer deadly outcomes.

Having purchased more than 10 cows during the past year was a potential risk factor for being a HM herd. To purchase animals may be a way of introducing new pathogens into the herd and colostrum from recently introduced dams might not contain antibodies protective against the herd specific pathogens. Purchases of animals are more likely in herds increasing quickly in size, or starting up. This factor was significant in the non-imputed dataset but with a high OR and wide confidence interval, an explanation could be due to a small number of observations for certain covariate combinations in the restricted dataset (the non-imputed data). In the imputed dataset the odds ratio and confidence interval for this variable were more reasonable.

4.4. Model "Hygiene"

As mentioned previously, finding a high average number of faecal pathogens when sampling calf groups at risk of having neonatal diarrhoea increased the odds of being a HM herd. This means that multiple pathogens were isolated more often from calves in HM than in LM herds. The presence of more than one enteropathogen has been suggested as one factor determining whether an infection results in a clinical or subclinal outcome (Reynolds et al., 1986; Hoet et al., 2003). The average number of faecal pathogens in high risk calf groups could be used in this way as a measure of a herd's pathogen load and as an indirect measure of hygiene in calf pens. It may therefore be useful when trying to motivate farmers to improve their hygiene routines.

An unexpected result was the association between use of maternity pen and type of herd, i.e. a reduced odds of being a HM herd if the maternity pens were used both for calving and for diseased cows. One explanation for this finding might be that HM herds in general used maternity pens more seldom. In our study, calvings in HM herds tended to occur more frequently in free stalls (>10% of calvings) ($p = 0.051$). This was not used as a practice; it was accidental when it happened. It is possible that calving in free stalls results in a greater number of calves that are abandoned, or that cross-suckling occurs, which can lead to reduced colostral intake making calves more susceptible to pathogens. In other studies, where most calves were born in free stalls, the suckling frequency was reduced and calf mortality in the first week of life was significantly increased (Streit and Ernst, 1992; Kjaestad and Simensen, 2001).

4.5. Other findings

As previously mentioned, enteritis has been found to be the most common cause of death during the first month of life, whereas death from respiratory disease is more common in older calves (Agerholm et al., 1993; Sivula et al., 1996b; Virtala et al., 1996; Svensson et al., 2006). The low median age at death among the HM herds in the present study (13 days) as compared with LM herds (24 days) therefore indicates that diarrhoea might be a cause of the elevated mortality risk in HM herds. In a study carried out on 63 Dutch dairy farms, the median age at occurrence of diarrhoea was 8 days (Perez et al., 1990). Waltner-Toews et al. (1986a) and Svensson et al. (2003) reported that the incidence of calf diarrhoea peaked during the second week after birth.

Salmonellosis is reported as one of the major infectious causes of calf mortality in North America (Maunsell and Donovan, 2008), but is rare in Sweden due to legislated control since 1961. With only two bulk milk samples with antibodies positive for *Salmonella* Dublin or *Salmonella* Typhimurium, one in each group, the higher calf mortality risk in HM herds is not likely to be explained by Salmonellosis. However a possible limitation of antibody tests on bulk milk from large dairy herds is that antibodies from a few infected cows may be diluted causing falsely negative results. In many countries, BVDV is endemic (Lindberg et al., 2006), and can be a major cause of impaired calf health (de Verdier Klingenberg et al., 1999). Due to the successful Swedish BVDV eradication programme, BVDV is, however, no longer a major contributor to calf mortality in Sweden. In the present study, two LM herds were positive for BVDV. They were not excluded from the study as they had a consistently twelve-month herd level calf mortality risk from the period September 2003–2006.

4.6. Statistical methods

The approach with initial univariable screening followed by multivariable analysis is fairly common when dealing with large datasets (Dohoo et al., 1997). Due to the limited sample size, we refrained from running a final model with all remaining variables, because this would have resulted in data being divided into too many groups with few observations. Of course, one weakness with this approach is that it was not possible to control for/investigate joint effects of the variables that were significant in the respective models. Some interactions or confounding factors may also have gone undetected.

Another aspect to bear in mind is that when dealing with large datasets the possibility of finding associations by chance alone increases, which means that associations between potential risk factors and type of herd are not necessarily directly or indirectly causal (Dohoo et al., 1997). This is also an inherent feature of the case–control study design, investigating associations rather than causality. Still, we believe that associations found were reasonable and can provide valuable input in the understanding of the multi-factorial background of calf mortality in large dairy herds.

5. Conclusions

The results of the study indicate that calf mortality in large dairy herds in Sweden has a multi-factorial background, and suggest that diarrhoea could be a major cause. Furthermore, our study indicates that feeding routines and fat-soluble vitamins might play an important role for calf health. Further studies of α-tocopherol and β-carotene and their association with calf mortality can provide new information useful when managing large dairy herds.

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