Enteropathogens and risk factors for diarrhea in Norwegian dairy calves

S. M. Gulliksen,*†[1] E. Jor,‡ K. I. Lie,* I. S. Hamnes,* T. Løken,* J. Åkerstedt,§ and O. Østerås*
*Norwegian School of Veterinary Science, PO Box 8146 Dep., NO-0033 Oslo, Norway
†TINE Norwegian Dairies, PO Box 58, NO-1431 Ås, Norway
‡National Veterinary Institute, PO Box 750, NO-0106 Oslo, Norway
§National Veterinary Institute Sandnes, PO Box 295, NO-4303 Sandnes, Norway

ABSTRACT

The aims of the current study were to estimate the prevalence of enteropathogens in calves in Norwegian dairy herds, evaluate the clinical consequences of protozoal infections, and identify risk factors for diarrhea. The 135 participating herds were randomly selected from those in The Norwegian Dairy Herd Recording System that had at least 15 cow-years. Each herd was followed for 1 yr. Fecal samples from calves with (n = 68) or without (n = 691) diarrhea were analyzed for the presence of *Cryptosporidium*, *Giardia*, and *Eimeria* species. Diarrheic samples (n = 191) were assayed for rotavirus group A, bovine coronavirus (BCoV), *Cryptosporidium*, and *Escherichia coli* F5 by antigen ELISA. Blood samples (n = 1,348) were analyzed for antibodies against BCoV and rotavirus. Potential risk factors for diarrhea were analyzed by using Cox regression analysis adjusted for herd frailty effect. Rotavirus and *Cryptosporidium* were the most commonly detected enteropathogens in diarrheic samples. A high level of *Cryptosporidium* shedding or BCoV seropositive calves in a herd was associated with an increased risk of diarrhea. Other factors found to increase the risk of diarrhea were use of slatted concrete floor in group pens versus other floor types [hazard ratio (HR) = 8.9], housing of calves in free-stalls compared with tie-stalls (HR = 3.7), purchasing of calves into the herd versus not purchasing calves (HR = 4.1), and calves being born during winter compared with other seasons of the year (HR = 1.5).
Key words: calf, enteropathogen, diarrhea, risk factor

INTRODUCTION

Enteric disease is a major health problem in calves, and diarrhea is associated with reduced weight gain and increased mortality rates in cattle production (Wittum et al., 1993; Virtala et al., 1996). Furthermore, diarrhea in young calves has been found to increase the risk of other diseases later in life (Van Donkersgoed et al., 1993). Diarrhea accounted for nearly 40% of all calf disease recordings in The Norwegian Dairy Herd Recording System (**NDHRS**) in 2005, with an incidence of 3.8%. The incidence of diarrhea was calculated to be 5.5% when adjusted for lack of recordings (Gulliksen et al., 2009).

Calves are at greatest risk of developing diarrhea during the first month of life, and the risk then decreases with age (Bendali et al., 1999; García et al., 2000). Rotavirus, bovine coronavirus (**BCoV**), *Escherichia coli* F5, and *Cryptosporidium* species are internationally recognized as the most important enteropathogens in acute diarrhea in young calves (Krogh and Henriksen, 1985; De Rycke et al., 1986; De la Fuente et al., 1999). Among the protozoa, species of *Eimeria* are considered relevant causes of diarrhea in calves beginning at approximately 3 wk of age (Svensson, 1993), whereas the importance of *Giardia intestinalis* as a cause of diarrhea in calves remains unclear (Björkman et al., 2003). Hamnes et al. (2006) concluded that both *Cryptosporidium* and *Giardia* species are widespread in Norwegian dairy herds, with herd prevalences of 53 and 93%, respectively, but did not investigate the association of these parasites with diarrhea. Bovine virus diarrhea virus (**BVDV**) is relevant as a cause of calf diarrhea in most countries (Werdin et al., 1989; Kelling et al., 2002), but BVDV has been eradicated in Norway, and freedom from this disease is monitored in a national surveillance and control program (Kampen et al., 2007).

The etiology of calf diarrhea is multifactorial and may include infective, environmental, nutritional, and management factors such as calves being born from a heifer (Clement et al., 1995), being born during the summer (Svensson et al., 2003, 2006), suckling (Svensson et al., 2003; Trotz-Williams et al., 2008a), low serum IgG concentrations (Blom, 1982), and large herd size (Frank and Kaneene, 1993).

The aims of the current study were to estimate the prevalence of selected enteropathogens in calves in dairy herds, evaluate the clinical consequences of protozoal infections, and identify risk factors for diarrhea.

MATERIALS AND METHODS

Study Herds

The study was designed as a longitudinal, cross-sectional survey, where a multistage sampling procedure (Gulliksen et al., 2008) was used to select NDHRS herds with at least 15 cow-years (days from first calving to culling within 1 yr/365 d) randomly from 30 veterinary districts throughout Norway. Altogether, 193 dairy herds were invited to participate in the study, of which 135 (69.9%) agreed to participate. These herds were divided geographically into 3 regions as follows: region 1 included eastern Norway (n = 62); region 2, western Norway (n = 23); and region 3, central and northern Norway (n = 50). Each herd participated for 1 yr. For logistical reasons, herds in region 1 were enrolled during autumn 2004, herds in region 2 during spring 2005, and herds in region 3 during autumn 2005. The overall study period lasted from September 1, 2004, to January 31, 2007.

Housing and Management

The herd owners were sent a questionnaire comprising 55 questions on animal housing, management, and feeding routines during the study year (Table 1). Altogether, 125 of the 135 (92.5%) participating farmers completed and returned the questionnaire.

Health Registrations

Health data were obtained from the NDHRS. Members of this system report diseases, treatments, and preventive treatments for each animal on a regular basis (Østerås et al., 2007). Each animal has an individual "health card," which follows the animal from birth until culling or slaughter. Calves intended for meat production have a common health card in the herd.

Participating herds received 12 health cards for registration of calf health events, one for each month, which were to be returned regardless of whether or not there was any information to register. In cases of disease for which a veterinarian was not consulted, the farmers were asked to record the events based upon definitions provided by the project. Diarrhea was defined as soft or watery feces lasting for 2 or more days, possibly in combination with impaired general condition or weight loss (Svensson et al., 2003).

Sampling

During the 2-yr period of fieldwork, a total of 64 local veterinarians were each responsible for between 1 to 14 herds, which they visited twice at approximately 6-mo intervals. On both calls, 12 calves under the age of 12 mo were randomly selected for sampling. The veterinarians were asked to sample the following age groups: 2 calves younger than 2 wk, 2 calves aged between 2 and 4 wk, 2 calves aged between 1 and 3 mo, 3 calves aged between 3 and 6 mo, and 3 calves aged between 6 and 12 mo. The veterinarians were instructed to collect approximately 2×10 g of feces and 10 mL of jugular blood from each calf. The samples were sent overnight to the National Veterinary Institute (Oslo, Norway) in a Styrofoam box with a cooling unit. The veterinarian classified the fecal consistency as diarrheic or normal based on visual appearance at the time of collection. For samples for which classification of the feces was missing, this was recorded by laboratory staff upon arrival of the sample at the laboratory. Serum was stored at –20°C until analysis.

Laboratory Examinations

For each herd, feces from the 8 youngest calves of at least 2 wk of age were examined for *Eimeria* oocysts by bright-field microscopy (Cornelissen et al., 1995), whereas feces from the 8 youngest animals of at least 1 wk of age were analyzed for *Cryptosporidium* oocysts and *Giardia* cysts by immunofluorescence microscopy (Hamnes et al., 2006; n = 691). For *Cryptosporidium* and *Giardia,* samples were classified into 4 groups depending on the number of cysts/oocysts found on average in each field of view at 400× magnification. The groups were defined as 0 (no cysts/oocysts), 1+ (1 cyst/oocyst), 2+ (2 to 10 cysts/oocysts), or 3+ (>10 cysts/oocysts). For *Eimeria*, 100× magnification was used. In case of insufficient amounts of feces sampled or lack of information on the calf, samples were substituted with feces from another calf of similar age in the same herd.

Diarrheic samples (n = 191) were examined for rotavirus group A, BCoV, *Cryptosporidium*, and *E. coli* F5 (K99) by an antigen ELISA (BIO K 071 from Bio-X Diagnostics Sprl, Jemelle, Belgium) according to the manufacturer's instructions.

Blood samples (n = 1,348) were assayed by ELISA for the presence of antibodies against BCoV (Svanovir BCV-Ab, Svanova Biotech AB, Uppsala, Sweden) and rotavirus group A (BIO K 126, Bio-X Diagnostics Sprl) as described by the producers. For rotavirus, ≥20% inhibition of the positive control was considered seropositive. To avoid interference from maternal antibodies, only samples from animals at least 150 d of age were tested.

Table 1. Variables on housing and management extracted from a questionnaire from 125 dairy herds in a survey on calf health in Norway between 2004 and 2007[1]

Variable	Class	Herds, N	Calves, n	Diarrhea cases, n (%)
Total		125	5,101	201 (3.9)
Stall type	Tie-stall	71	2,195	56 (2.6)
	Free-stall	54	2,906	145 (5.0)
Purchase of calves during the last year	Yes	25	1,187	95 (8.0)
	No	95	3,712	94 (2.5)
	No information	5	202	12 (5.9)
Calving facilities	Tie-stall: in box	71	2,195	56 (2.6)
	Free-stall: cubicle section or alley area	19	916	15 (1.6)
	Free-stall: calving pen	34	1,954	130 (6.6)
	No information	1	36	0 (0.0)
Separation from dam after calving	>24 h	10	430	21 (4.9)
	≤24 h	114	4,635	180 (3.9)
	No information	1	36	0 (0.0)
Suckling as feeding regime	No	112	4,500	177 (3.9)
	Yes	13	601	24 (4.0)
First feeding of colostrum after birth	Within 30 min	35	1,112	46 (4.1)
	Within 2 h	51	2,226	88 (4.0)
	Within 4 h	12	464	40 (8.6)
	Later	25	1,161	26 (2.2)
	No information	2	138	1 (0.6)
Minimum colostrum fed first feeding	≤1 L	22	984	54 (5.5)
	1–2 L	81	3,068	52 (1.7)
	>2 L	14	673	68 (10.1)
	No information	8	376	27 (7.2)
Housing during first week of life	Room shared with cows	98	3,801	164 (4.3)
	Separate room for calves	25	1,235	26 (2.1)
	No information	2	65	11 (16.9)
Use of heat lamp	Always	25	1,131	59 (5.2)
	Occasionally or never	99	3,898	142 (3.6)
	No information	1	72	0 (0.0)
Confinement from two weeks of age	Single pen	60	2,317	142 (6.1)
	Group pen	64	2,761	59 (2.1)
	No information	1	23	0 (0.0)
Straw in single pen	Yes	64	2,597	136 (5.2)
	No	36	1,264	37 (2.9)
	No information	25	1,240	28 (2.3)
Removal of manure in single pen less than once weekly	Yes	27	1,115	103 (9.2)
	No	69	2,607	51 (2.0)
	No information	29	1,289	47 (3.6)
Slatted concrete floor in group pen	Yes	69	2,739	147 (5.4)
	No	49	2,157	53 (2.5)
	No information	7	205	1 (0.5)
Deep litter in resting area in group pen	Yes	10	347	28 (8.1)
	No	111	4,653	172 (3.7)
	No information	4	101	1 (1.0)
Maximum age difference between youngest and oldest calf in group pen	>8 wk	25	800	41 (5.1)
	≤8 wk	89	3,957	158 (4.0)
	No information	11	344	2 (0.6)
Feeding with sour milk	Yes	46	1,628	38 (2.3)
	No	61	2,589	143 (5.5)
	No information	18	884	20 (2.3)
Volume milk fed per day, wk 1	≤4 L	43	1,671	50 (3.0)
	4.01–6 L	37	1,543	119 (7.7)
	6.01–10 L	9	412	15 (3.6)
	Ad libitum/automatic	33	1,290	16 (1.2)
	No information	3	185	1 (0.5)
Volume milk fed per day, wk 2	≤4 L	39	1,691	88 (5.2)
	4.01–6 L	27	1,087	47 (4.3)
	6.0–10 L	10	475	22 (4.6)
	Ad libitum/automatic	46	1,663	43 (2.6)
	No information	3	185	1 (0.5)

Continued

Table 1 (Continued). Variables on housing and management extracted from a questionnaire from 125 dairy herds in a survey on calf health in Norway between 2004 and 2007[1]

Variable	Class	Herds, N	Calves, n	Diarrhea cases, n (%)
Volume milk fed per day, wk 3	≤4 L	31	1,358	94 (6.9)
	4.01–6 L	21	824	29 (3.5)
	6.01–10 L	10	524	23 (4.4)
	Ad libitum/automatic	60	2,210	54 (2.4)
	No information	3	185	1 (0.5)
Volume milk fed per day, wk 4	≤4 L	27	1,106	63 (5.7)
	4.01–6 L	17	653	20 (3.1)
	6.01–10 L	9	438	21 (4.8)
	Ad libitum/automatic	69	2,719	96 (3.5)
	No information	3	185	1 (0.5)
Volume milk fed per day, >4 wk of age	≤4 L	29	1,259	70 (5.6)
	4.01–6 L	10	364	11 (3.0)
	6.01–10 L	7	318	21 (6.6)
	Ad libitum/automatic	71	2,637	74 (2.8)
	No information	8	523	25 (4.8)

[1]Only calves born and raised in the same herd are included. Reported cases of diarrhea included.

Statistical Analysis

Calves born in the participating 135 herds during the project period were included in the study (n = 5,621). For descriptive statistics and statistical analysis, relevant NDHRS data were extracted and transferred, together with data recorded by the project, to SAS version 9.1 (SAS Institute Inc., Cary, NC). Four separate Cox proportional hazard models (Cox, 1972) were fitted by using the PROC PHREG statement with diarrhea (0/1) as dependent variable based on recordings in the NDHRS.

In the first model, excretion levels of *Cryptosporidium* oocysts, *Giardia* cysts, and *Eimeria* oocysts, and percentage of BCoV or rotavirus seropositive calves among the sampled calves in a herd were the only fixed effects included. Excretion level in a herd was calculated as the number of positive samples divided by the number of calves sampled. A binary variable was created for herds with (1) or without (0) any calves excreting (oo)cysts. Hierarchical dummy variables for excretion levels were then created. To obtain roughly equal numbers of animals in each category, the 10th, 25th, 50th, 75th, and 90th percentiles of proportion of excreting calves among the sampled calves per herd were used. For *Cryptosporidium,* the percentiles on herd level corresponded to >12.5, >25, >37, >50, and >62% of the sampled calves excreting oocysts. The equivalent percentiles for *Giardia* were >25, >50, >62, >83, and >87%, and for *Eimeria* >12.5, >37, >50, >75, and >87%, respectively. For BCoV, the corresponding percentiles for seropositive calves were >10, >33, >72, and >90%, and for rotavirus >40, >50, >67, and >82%.

In the second model, the influence of housing and management (Table 1) on the occurrence of diarrhea

was studied. Herds without a completed questionnaire were excluded (n = 10), leaving 5,101 calves in the study population. Separate groups were created for missing values of all variables. A season variable was created for each calf based on the month of birth. Spring was defined as March through May, summer as June through August, autumn as September through November, and winter as December through February. Hierarchical dummy variables were created for herd size divided into >20 cow-years, >50 cow-years, and >70 cow-years.

In the third model, the significant variables from the first and second models were combined. Variables on commitment to calf health recordings were included in the final model to adjust for the difference in reported diarrhea incidence between herds because of lack of recordings.

In the fourth model, all missing values were deleted, leaving a study population of 2,703 calves from 71 herds. Variables included in the second and third model were tested.

The positive stable frailty models in the SAS macro (Shu and Klein, 1999, 2005) were included in models 1 to 3. The significance of the frailty effect was assessed by the likelihood ratio test of independence [H_0: theta $(\theta) = 1$]. The frailty effect equals the strength of association between 2 individuals within the same herd, measured by Kendall's τ $(1 - \theta)$, and was considered significant at $P < 0.05$. The end of the observation period was defined as 180 d of age for a calf that had no recorded event of diarrhea. Data were censored when the animal left the herd due to sale or slaughter. Only the first case of diarrhea from birth until 180 d of age was considered an event. The significance of the different variables was initially evaluated by univari-

Table 2. Diarrheic and normal feces from calves in 135 Norwegian dairy herds examined for protozoa by bright field microscopy (*Eimeria*) and immunofluorescent staining (*Cryptosporidium* and *Giardia*)

Result	Diarrheic samples (n = 68)			Normal samples (n = 691)		
	Cryptosporidium (%)	*Eimeria* (%)	*Giardia* (%)	*Cryptosporidium* (%)	*Eimeria* (%)	*Giardia* (%)
Negative	42 (61.7)	39 (57.4)	25 (36.8)	463 (67.0)	338 (48.9)	226 (32.7)
1+	24 (35.3)	20 (29.4)	39 (57.4)	208 (30.1)	250 (36.2)	404 (58.5)
2+	1 (1.5)	4 (5.9)	2 (2.9)	15 (2.2)	72 (10.4)	26 (3.8)
3+	1 (1.5)	5 (7.3)	2 (2.9)	5 (0.7)	31 (4.5)	35 (5.0)

ate analysis. If the *P*-value was <0.1, the variable was included in a final multivariable model. Nonsignificant variables were removed one by one by using backward stepwise elimination with inclusion criteria of $P < 0.05$. Possible interactions between significant fixed effects were tested.

Based on the significant hierarchical dummy variables included in model 1, binary variables on herd level were created for herds with >12.5% of the sampled calves excreting *Cryptosporidium* oocysts (1) and herds with ≤12.5% of the sampled calves excreting oocysts (0). Corresponding binary variables on the herd level were created for rotavirus and BCoV [more than (1) or equal or less than (0) 67 and 72% seropositive calves among the sampled, respectively]. PROC GENMOD with binomial distribution and logit link function with Wald's statistic type-3 contrasts was used to test the influence of housing and management on infectious status

in the herds. Because of the nature of the fixed effects, these analyses were performed at the herd level. Nonsignificant variables were removed one by one by using backward stepwise elimination, with inclusion criteria of $P < 0.05$. The model fit was evaluated by using -2 log-likelihood (Dohoo et al., 2003).

RESULTS

Enteropathogens

In total, 191 of 4,041 (4.7%) fecal samples were classified as diarrheic (Figure 1). *Cryptosporidium* was the only protozoan more prevalent in diarrheic samples than in normal samples (Table 2). In the diarrheic samples analyzed by microscopy (n = 68), both *Cryptosporidium* and *Giardia* were found in 10 (14.7%), *Eimeria* and *Giardia* were found in 9 (13.2%), whereas *Cryptosporidium*
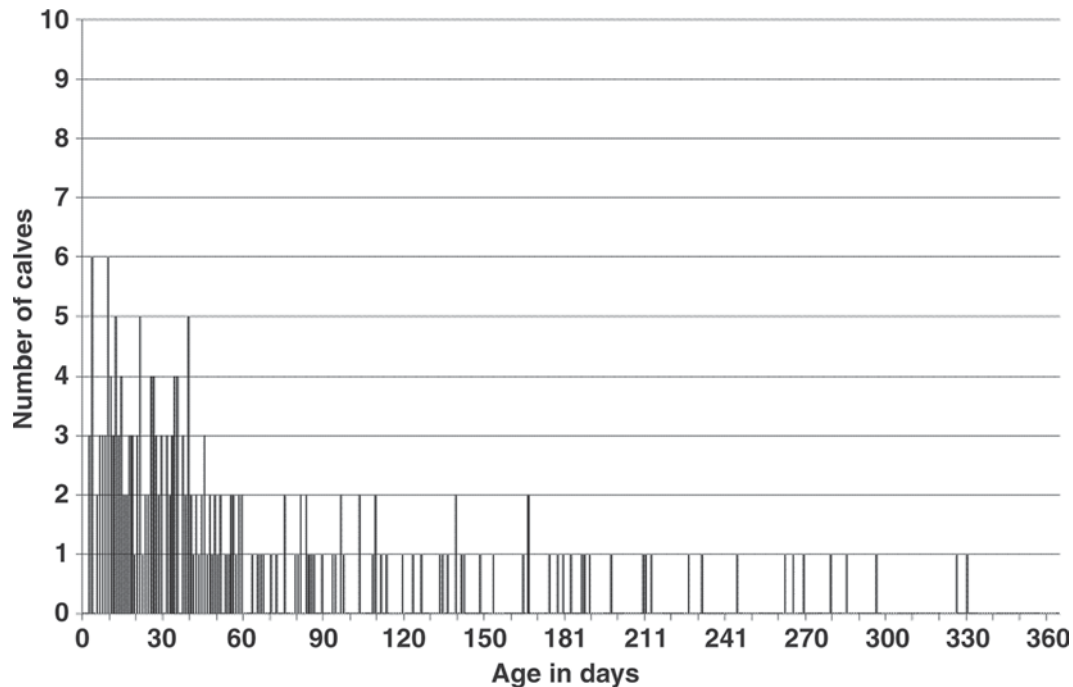

Figure 1. Age of calves (n = 191) at sampling in which the fecal sample was considered to be diarrheic. Initiation or duration of diarrhea is not indicated. The sampling was performed in 135 dairy herds participating in a survey of calf health in Norway between 2004 and 2007.

Table 3. Hazard ratio (HR; with 95% confidence interval [CI]) estimates for diarrhea or not (0/1) using the Cox regression model accounting for cluster effects at the herd-level in 5,101 calves in 125 Norwegian dairy herds[1]

Fixed effect	Class	Herds, N	Calves, n	Estimate (β)	SE	HR (95% CI)	P-value
Recorded >50% of all dehornings	Yes	33	1,228	2.54	0.60	12.7 (3.8–42.0)	<0.001
	No	92	3,873	—	—	1.0	—
Stall type	Free-stall	54	2,195	1.30	0.60	3.7 (1.1–12.2)	<0.05
	Tie-stall	71	2,906	—	—	1.0	—
Purchase of calves during the last year	Yes	25	1,187	1.42	0.56	4.1 (1.4–12.4)	<0.05
	No	95	3,712	—	—	1.0	—
	No information	5	202	—	—	—	NS
Slatted concrete floor in group pen	Yes	69	2,739	2.17	0.68	8.9 (2.3–33.1)	<0.05
	No	49	2,157	—	—	1.0	—
	No information	7	205	—	—	—	NS
Born during winter	Yes		1,189	0.42	0.15	1.5 (1.1–2.0)	<0.001
	No		3,912	—	—	1.0	—
Born from purchased dam	Yes		4,362	−0.68	0.37	0.5 (0.2–1.1)	0.07
	No		739	—	—	1.0	—
θ (1 − Kendall's τ)	Herds	125		0.60	0.07	—	<0.001

[1]The cluster effect within herd is assessed by the frailty effect (Kendall's τ).

and *Eimeria* were found in 6 (8.8%), and all 3 parasites were found in 6 (8.8%). In normal fecal samples (n = 691), both *Cryptosporidium* and *Giardia* were found in 77 (11.1%), *Eimeria* and *Giardia* were found in 157 (22.7%), whereas *Cryptosporidium* and *Eimeria* were found in 28 (4.1%), and all 3 parasites were found in 88 (12.7%).

In the 191 diarrhea samples examined by antigen-ELISA, rotavirus and *Cryptosporidium* were detected in 9.9 and 4.2%, respectively, whereas *E. coli* F5 was detected in 5 (2.6%) samples; BCoV was not detected in any sample. By antigen-ELISA, no enteropathogens were found in 162 of 191 (84.8%) diarrheic samples. Diarrhea was recorded at least once in 201 of 5,101 (3.9%) calves in the NDHRS during the project period. In total, 530 of 1,348 (39.3%) calves were seropositive for BCoV, whereas 912 (67.7%) were seropositive for rotavirus.

Risk Factors for Clinical Diarrhea

Analyses including only enteropathogens revealed that calves born in herds with >72% of the sampled calves being seropositive for BCoV had an increased risk of diarrhea [hazard ratio (**HR**)] of 4.5 [95% confidence interval (**CI**): 1.3–15.6]. This was also true for calves from herds in which >67% of the sampled calves were seropositive for rotavirus and calves from herds with more than 12.5% calves excreting *Cryptosporidium* oocysts (HR = 3.5; 95% CI: 2.5–4.9 and HR = 2.4; 95% CI: 1.4–4.2, respectively), although HR was not significant when accounting for clustering. Associations between other enteropathogens and diarrhea were not found at either the herd level or the calf level.

Factors found to be associated with an increased risk of diarrhea were slatted concrete floor in group pens versus other floor types (HR = 8.9), being housed in

free-stalls compared with tie-stalls (HR = 3.7), being in a herd in which calves were purchased versus being in a herd that did not purchase calves (HR = 4.1), and being born during winter compared with other seasons of the year (HR = 1.5) (Table 3). The associations between different risk factors and diarrhea without accounting for cluster effects at the herd level are presented in Table 4.

Calves housed in group pens with slatted concrete floors had a borderline increased risk of shedding *Cryptosporidium* oocysts (odds ratio = 2.3, 95% CI: 0.87–6.0, P = 0.09) compared with calves housed on solid floor or deep litter.

DISCUSSION

Enteropathogens

In general, the prevalence of the different enteropathogens was low compared with reports from other countries (De la Fuente et al., 1999; García et al., 2000; Uhde et al., 2008). Most likely, not all calves reported to have diarrhea had impaired general condition at the time of sampling. Several of the diarrheic cases might have been short term and the symptoms were possibly caused by malnutrition or rapid change in diet. Also, some samples could be false negatives, especially for pathogens with low or intermittent shedding. Furthermore, other pathogenic bacteria as well as viruses or protozoa may cause diarrhea. Because of a national eradication program, Norway was declared free from BVDV in 2006 (Kampen et al., 2007). *Salmonella* spp. are rarely detected in Norwegian cattle (Lyngstad et al., 2007). Recently, more than 500 fecal samples from 37 dairy herds with various calf health problems were analyzed for *Salmonella*, and all samples were negative (S. M. Gulliksen, K. I. Lie, E. Jor, and O. Østerås;

Table 4. Hazard ratio (HR; with 95% confidence interval [CI]) estimates for diarrhea or not (0/1) using the Cox regression model without accounting for cluster effects at the herd level in 5,101 calves in 125 Norwegian dairy herds

Fixed effect	Class	Herds, N	Calves, n	Estimate (β)	SE	HR (95% CI)	*P*-value
Confirming complete calf health records	Yes	62	2,522	0.92	0.19	2.5 (1.7–3.7)	<0.001
	No	63	2,579	—	—	1.0	—
Recorded >50% of all dehornings	Yes	33	1,228	0.40	0.17	1.5 (1.1–2.1)	<0.05
	No	92	3,873	—	—	1.0	—
Stall type	Free-stall	54	2,195	0.81	0.18	2.3 (1.6–3.2)	<0.001
	Tie-stall	71	2,906	—	—	1.0	—
Herd size (cow-years)	>70	5	566	1.22	0.24	3.4 (2.1–5.4)	<0.001
	≤70	120	4,535	—	—	1.0	—
Purchase of calves during the last year	Yes	25	1,187	0.41	0.17	1.5 (1.1–2.1)	<0.05
	No information	5	202	1.23	0.33	3.3 (1.7–6.4)	<0.001
	No	95	3,712	—	—	1.0	—
Straw in single pen	Yes	64	2,597	1.07	0.19	2.9 (2.0–4.3)	<0.001
	No	36	1,264	—	—	1.0	—
	No information	25	1,240	—	—	—	NS
Removal of manure in single pen less than once weekly	Yes	27	1,115	1.41	0.21	4.1 (2.7–6.1)	<0.001
	No information	29	1,289	0.78	0.25	2.2 (1.4–3.5)	<0.05
	No	69	2,607	—	—	1.0	—
Slatted concrete floor in group pen	Yes	69	2,739	1.10	0.19	3.0 (2.0–4.4)	<0.001
	No	49	2,157	—	—	1.0	—
	No information	7	205	—	—	—	NS
Deep litter in resting area in group pen	Yes	10	347	1.23	0.23	3.4 (2.2–5.4)	<0.001
	No	111	4,653	—	—	1.0	—
	No information	4	101	—	—	—	NS
Proportion of sampled calves excreting *Cryptosporidium* oocysts	>12.5%	25	1,005	0.59	0.30	1.8 (1.0–3.3)	0.05
	≤12.5%	100	4,096	—	—	1.0	—
Born during winter	Yes		1,189	0.63	0.15	1.9 (1.4–2.5)	<0.001
	No		3,912	—	—	1.0	—
Born from purchased dam	Yes		4,362	−1.05	0.35	0.35 (0.2–0.7)	<0.05
	No		739	—	—	1.0	—

unpublished data). The isolated geographic location of Norway, together with efficient surveillance and restrictive rules regarding import and trade of animals and animal products have contributed to the freedom from several infectious diseases that Norway benefits from today.

That rotavirus and *Cryptosporidium* were the most frequently detected enteropathogens in diarrheic samples concurs with results from studies from other European countries (De la Fuente et al., 1999; Björkman et al., 2003; Uhde et al., 2008). Based on the serological results, both BCoV and rotavirus are common in Norwegian dairy herds. A high number of seropositive calves suggests an active or newly introduced infection in a herd, hence an increased risk of diarrhea. Low sensitivity of the antigen-ELISA for BCoV may explain why animals shedding BCoV were not detected. Interestingly, real-time PCR results from a recent Norwegian study (E. Jor, National Veterinary Institute, Oslo, Norway; personal communication) support the negative results from the antigen-ELISA.

The direct assessment of clinical consequence of the different enteropathogens was restricted to protozoal infections because microscopy was the only analysis performed on both diarrheic and normal samples. Among the protozoa, *Cryptosporidium* was the only agent more prevalent in diarrheic samples than in normal samples. Furthermore, a high number of calves shedding *Cryptosporidium* in a herd was associated with an increased risk of diarrhea. This is in accordance with Trotz-Williams et al. (2007) who found that calves shedding *Cryptosporidium* oocysts had an odds ratio of 5.3 of diarrhea compared with nonshedding calves. Only 50% of the diarrheic samples were analyzed by both antigen-ELISA and immunofluorescence microscopy because of the limited amounts of feces sampled. A considerably larger proportion of the samples were found positive for *Cryptosporidium* by the latter method (4.4 vs. 38.3%, respectively). As a diagnostic test, the cut-off of the antigen-ELISA was probably set to discriminate between clinically relevant and irrelevant numbers of oocysts. Thus, samples containing small numbers of oocysts might have been classified as negative by this test. Immunofluorescence microscopy is a more sensitive and specific method compared with the antigen-ELISA. In addition, it has the advantage of quantifying the level of shedding compared with the ELISA, which only provides a positive or negative result. Trotz-Williams et al. (2008b) found a prevalence of 30% of *Cryptosporidium* shedding calves in their study, using microscopic slide flotation, which corresponds to 33 and 38.3% in normal and diarrheic samples, respectively, found in the current study.

The presence of 2 or more enteropathogens simultaneously has been suggested to result in the clinical development of infection usually being worse (Reynolds et al., 1986; Hoet et al., 2003). However, concurrent infection with both *Cryptosporidium* and *Eimeria* was the only combination found more frequently in diarrheic samples than in normal samples. Other mixed infections with protozoa were equally common in diarrheic samples as normal samples. This could be, in part, because a fecal sample from a calf found to be normal on the day of sampling might have been diarrheic before or after sampling. Calves that previously had diarrhea but returned to normal on the day of sampling could still be shedding oocysts or cysts, making it appear as if these organisms were isolated from normal feces. The age distribution of the diarrheic samples corresponds to the distribution of clinically reported first cases of diarrhea in the NDHRS (Gulliksen et al., 2009).

Risk Factors for Clinical Diarrhea

The results of this study showed an increased risk of clinical diarrhea, as reported in the NDHRS, in herds exceeding 70 cow-years. Free-stall compared with tie-stall housing systems increase contact between animals, and in larger herds, cows and calves may be more densely housed, which could promote the spread of infections and increase environmental contamination. Trotz-Williams et al. (2008b) found a trend ($P = 0.09$) of increased risk of calves shedding *Cryptosporidium* in larger farms, and the possibility of large outbreak problems will be greater in a larger population (Frank and Kaneene, 1993). As herd size increases, more mechanization and technological developments are used, the time physically spent by the farmer in the barn decreases, and the daily inspection of each individual is reduced. Lance et al. (1992) found the lack of individual attention in large herds to be important in explaining high mortality rates in preweaned heifers, with diarrhea and dehydration as the major reasons for death.

Purchase of calves was found to be a risk factor for diarrhea, probably because of the introduction of new infectious agents (Van Schaik et al., 2002). Interestingly, calves born to purchased dams were found to have a lower risk of diarrhea than calves born to dams raised in the herd. This finding might indicate that calves born to purchased cows are protected against newly introduced infectious agents through passive immunity, whereas the other calves are not. On the other hand, regular introduction of susceptible animals could contribute to maintaining infections with enteropathogens already circulating in the farm. The association between risk of diarrhea and being born to a purchased

dam or in a herd that purchased calves was not significant when all missing values were removed from the model.

Removing bedding in single boxes at least once weekly resulted in a lower risk of diarrhea. Removal of bedding could reduce the occurrence of pathogens, and previous studies have concluded that cleaning is an important management factor in preventing high levels of *Cryptosporidium* oocysts (Mohammed et al., 1999; Hamnes et al., 2006; Maddox-Hyttel et al., 2006).

Several studies have shown that calves kept on deep litter have an increased risk of diarrhea (Svensson et al., 2006) and infections with enteropathogens (Mohammed et al., 1999; Jäger et al., 2005). This contrasts with the result of the present study, in which both the risk of shedding *Cryptosporidium* oocysts and the risk of diarrhea were increased in calves kept on slatted concrete floors compared with other types of flooring. Because one would expect solid floors to be more dirty than slatted floors if not scraped and cleaned properly, this finding is somewhat difficult to explain. Draft and local chilling from the dung basement through the slatted floor could be part of the explanation, as draft has been shown to be associated with the risk of infectious diseases in calves from 0 to 90 d of age (Lundborg et al., 2005). Local temperature, ammonia concentration, and pH have an effect on the survival of *Cryptosporidium* oocysts (Reinoso et al., 2008), and these are all factors likely to be influenced by floor type. Further research is needed in this area.

The finding that the greatest risk of diarrhea is in calves born during the winter months is consistent with results from the study of Frank and Kaneene (1993), but contrasts with results from Swedish studies (Svensson et al., 2003, 2006). An increased risk of diarrhea during winter might be caused by higher levels of infectious agents during this season due to higher animal density per housing unit, lower ambient temperature, and higher humidity of inside air. A cool, humid environment supports the survival of several infectious agents for extended periods (Fayer et al., 2005). Maddox-Hyttel et al. (2006) found higher excretion levels of *Cryptosporidium* oocysts during winter than during summer, as did Hamnes et al. (2006). Furthermore, Norwegian dairy cows calving during winter produce colostrum of poorer quality than that produced by cows calving during any other season of the year (Gulliksen et al., 2008). The importance of adequate consumption of colostrum by calves and its effect on calf health is well recognized. Hence, intake of colostrum with a lower concentration of IgG could further increase the risk of diarrhea in calves born during this season. However, no variables, including colostrum feeding routines, were found to influence the risk of diarrhea in this study (Table 1).

The herd-level cluster effect was extremely high in the current study (Kendall's $\tau = 0.40$). This may be caused by varying commitment to calf health recordings and the effect of management factors being highly dependent on other features specific for each herd, such as dietary or environmental factors, in addition to general infection status. The validity of the reporting of calf health events in the NDHRS has been assessed elsewhere (Gulliksen et al., 2009), in which herds with complete calf health records and those reporting more than 50% of all dehornings have been found to report a significantly higher incidence of calf diarrhea.

Of the enteropathogens investigated in this study, rotavirus and *Cryptosporidium* appear to be the most frequently associated with calf diarrhea in Norwegian dairy herds. However, the overall prevalence of enteropathogens in diarrheic samples was low, which may indicate that other etiological agents are important. Control of livestock trading and preventive hygiene measures are generally considered important in reducing the incidence of infectious calf diarrhea. As dairy cattle farming moves toward larger herd sizes and novel housing systems, new challenges in calf management should be prioritized to avoid increasing disease rates. Further studies on the clinical importance of rotavirus and BCoV (as well as other enteropathogens) and the associations between feeding and calf diarrhea should be conducted.

ACKNOWLEDGMENTS

REFERENCES

Bendali, F., H. Bichet, F. Schelcher, and M. Sanaa. 1999. Pattern of diarrhoea in newborn beef calves in south-west France. Vet. Res. 30:61–74.

Björkman, C., C. Svensson, B. Christensson, and K. de Verdier. 2003. *Cryptosporidium parvum* and *Giardia intestinali*s in calf diarrhoea in Sweden. Acta Vet. Scand. 44:145–152.

Blom, J. Y. 1982. The relationship between serum immunoglobulin values and incidence of respiratory disease and enteritis in calves. Nord. Vet. Med. 34:276–284.

Clement, J. C., M. E. King, M. D. Salman, T. E. Wittum, H. H. Casper, and K. G. Odde. 1995. Use of epidemiologic principles to identify risk factors associated with the development of diarrhea in calves in five beef herds. J. Am. Vet. Med. Assoc. 207:1334–1338.

Cornelissen, A. W., R. Verstegen, H. Van den Brand, N. M. Perie, M. Eysker, T. J. Lam, and A. Pijpers. 1995. An observational study

of *Eimeria* species in housed cattle on Dutch dairy farms. Vet. Parasitol. 56:7–16.

Cox, D. R. 1972. Regression models and life-tables (with discussion). J. R. Stat. Soc., B 34:187–220.

De la Fuente, R., M. Luzon, J. A. Ruiz-Santa-Quiteria, A. Garcia, D. Cid, J. A. Orden, S. Garcia, R. Sanz, and M. Gomez-Bautista. 1999. *Cryptosporidium* and concurrent infections with other major enteropathogens in 1 to 30-day-old diarrheic dairy calves in central Spain. Vet. Parasitol. 80:179–185.

De Rycke, J., S. Bernard, J. Laporte, M. Naciri, M. R. Popoff, and A. Rodolakis. 1986. Prevalence of various enteropathogens in the feces of diarrheic and healthy calves. Ann. Rech. Vet. 17:159–168.

Dohoo, I., W. Martin, and H. Stryhn. 2003. Veterinary epidemiologic research. 1st ed. S. M. McPike, ed. AVC Inc., Charlottetown, PEI, Canada

Fayer, R., M. Santin, and L. Xiao. 2005. *Cryptosporidium bovis* n. sp. (Apicomplexa: *Cryptosporidiidae*) in cattle (*Bos taurus*). J. Parasitol. 91:624–629.

Frank, N. A., and J. B. Kaneene. 1993. Management risk factors associated with calf diarrhea in Michigan dairy herds. J. Dairy Sci. 76:1313–1323.

García, A., J. A. Ruiz-Santa-Quiteria, J. A. Orden, D. Cid, R. Sanz, M. Gomez-Bautista, and R. De la Fuente. 2000. Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comp. Immunol. Microbiol. Infect. Dis. 23:175–183.

Gulliksen, S. M., K. I. Lie, and O. Østerås. 2009. Calf health monitoring in Norwegian dairy herds. J. Dairy Sci. 92:1660–1669.

Gulliksen, S. M., K. I. Lie, L. Sølverød, and O. Østerås. 2008. Risk factors associated with colostrum quality in Norwegian dairy cows. J. Dairy Sci. 91:704–712.

Hamnes, I. S., B. Gjerde, and L. Robertson. 2006. Prevalence of *Giardia* and *Cryptosporidium* in dairy calves in three areas of Norway. Vet. Parasitol. 140:204–216.

Hoet, A. E., P. R. Nielsen, M. Hasoksuz, C. Thomas, T. E. Wittum, and L. J. Saif. 2003. Detection of bovine torovirus and other enteric pathogens in feces from diarrhea cases in cattle. J. Vet. Diagn. Invest. 15:205–212.

Jäger, M., M. Gauly, C. Bauer, K. Failing, G. Erhardt, and H. Zahner. 2005. Endoparasites in calves of beef cattle herds: Management systems dependent and genetic influences. Vet. Parasitol. 131:173–191.

Kampen, A. H., J. Åkerstedt, S. Gudmundsson, P. Hopp, G. Grøneng, and O. Nyberg. 2007. The surveillance and control programme for bovine virus diarrhoea (BVD) in Norway. Pages 65–71 in Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway—Annual report 2006. E. Brun, ed. The National Veterinary Institute, Oslo, Norway.

Kelling, C. L., D. J. Steffen, V. L. Cooper, D. S. Higuchi, and K. M. Eskridge. 2002. Effect of infection with bovine viral diarrhea virus alone, bovine rotavirus alone, or concurrent infection with both on enteric disease in gnotobiotic neonatal calves. Am. J. Vet. Res. 63:1179–1186.

Krogh, H. V., and S. A. Henriksen. 1985. Bovine cryptosporidiosis in Denmark. 2. Cryptosporidia associated with neonatal calf diarrhea. Nord. Vet. Med. 37:42–47.

Lance, S. E., G. Y. Miller, D. D. Hancock, P. C. Bartlett, L. E. Heider, and M. L. Moeschberger. 1992. Effects of environment and management on mortality in preweaned dairy calves. J. Am. Vet. Med. Assoc. 201:1197–1202.

Lundborg, G. K., E. C. Svensson, and P. A. Oltenacu. 2005. Herd-level risk factors for infectious diseases in Swedish dairy calves aged 0–90 days. Prev. Vet. Med. 68:123–143.

Lyngstad, T. M., P. Hopp, M. Hofshagen, B. Bergsjø, T. Bruheim, O. Eikenæs, K. Flesjå, and B. Øvsthus. 2007. The surveillance and control programmes for *Salmonella* in live animals, eggs and meat in Norway. Pages 21–28 in Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway—Annual report 2006. E. Brun, ed. The National Veterinary Institute, Oslo, Norway.

Maddox-Hyttel, C., R. B. Langkjær, H. L. Enemark, and H. Vigre. 2006. *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs—Occurrence and management associated risk factors. Vet. Parasitol. 141:48–59.

Mohammed, H. O., S. E. Wade, and S. Schaaf. 1999. Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. Vet. Parasitol. 83:1–13.

Østerås, O., H. Solbu, A. O. Refsdal, T. Roalkvam, O. Filseth, and A. Minsaas. 2007. Results and evaluation of thirty years of health recordings in the Norwegian dairy cattle population. J. Dairy Sci. 90:4483–4497.

Reinoso, R., E. Becares, and H. V. Smith. 2008. Effect of various environmental factors on the viability of *Cryptosporidium parvum* oocysts. J. Appl. Microbiol. 104:980–986.

Reynolds, D. J., J. H. Morgan, N. Chanter, P. W. Jones, J. C. Bridger, T. G. Debney, and K. J. Bunch. 1986. Microbiology of calf diarrhoea in southern Britain. Vet. Rec. 119:34–39.

Shu, Y., and J. P. Klein. 1999. A SAS Macro for the Positive Stable Frailty Model. Pages 47–52 in Proc. Am. Stat. Assoc., Statistical Computing Section, Am. Stat. Assoc., Alexandria, VA.

Shu, Y., and J. P. Klein. 2005. A SAS Macro for the Positive Stable Frailty Model. http://www.biostat.mcw.edu/software/SoftMenu.html Accessed August 23, 2008.

Svensson, C. 1993. Peripartal excretion of *Eimeria* oocyst by cows on Swedish dairy farms and the age of calves at first excretion. Acta Vet. Scand. 34:77–81.

Svensson, C., J. Hultgren, and P. A. Oltenacu. 2006. Morbidity in 3–7-month-old dairy calves in south-western Sweden, and risk factors for diarrhoea and respiratory disease. Prev. Vet. Med. 74:162–179.

Svensson, C., K. Lundborg, U. Emanuelson, and S. O. Olsson. 2003. Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Prev. Vet. Med. 58:179–197.

Trotz-Williams, L. A., K. E. Leslie, and A. S. Peregrine. 2008a. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. J. Dairy Sci. 91:3840–3849.

Trotz-Williams, L. A., S. W. Martin, K. E. Leslie, T. Duffield, D. V. Nydam, and A. S. Peregrine. 2008b. Association between management practices and within-herd prevalence of *Cryptosporidium parvum* shedding on dairy farms in southern Ontario. Prev. Vet. Med. 83:11–23.

Trotz-Williams, L. A., M. S. Wayne, K. E. Leslie, T. Duffield, D. V. Nydam, and A. S. Peregrine. 2007. Calf-level risk factors for neonatal diarrhea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. Prev. Vet. Med. 82:12–28.

Uhde, F. L., T. Kaufmann, H. Sager, S. Albini, R. Zanoni, E. Schelling, and M. Meylan. 2008. Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. Vet. Rec. 163:362–366.

Van Donkersgoed, J., C. S. Ribble, L. G. Boyer, and H. G. Townsend. 1993. Epidemiological study of enzootic pneumonia in dairy calves in Saskatchewan. Can. J. Vet. Res. 57:247–254.

Van Schaik, G., Y. H. Schukken, M. Nielen, A. A. Dijkhuizen, H. W. Barkema, and G. Benedictus. 2002. Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: A cohort study. Prev. Vet. Med. 54:279–289.

Virtala, A. M., G. D. Mechor, Y. T. Grohn, and H. N. Erb. 1996. Morbidity from nonrespiratory diseases and mortality in dairy heifers during the first three months of life. J. Am. Vet. Med. Assoc. 208:2043–2046.

Werdin, R. E., T. R. Ames, S. M. Goyal, and G. P. DeVries. 1989. Diagnostic investigation of bovine viral diarrhea infection in a Minnesota dairy herd. J. Vet. Diagn. Invest. 1:57–61.

Wittum, T. E., M. D. Salman, K. G. Odde, R. G. Mortimer, and M. E. King. 1993. Causes and costs of calf mortality in Colorado beef herds participating in the National Animal Health Monitoring System. J. Am. Vet. Med. Assoc. 203:232–236.