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; Garcia 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

| 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: cubic 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)             |
| 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)              |
| 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 (10.9)             |
| 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       | 3,937     | 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)              |
|                                              | 6.01–10 L                      | 10       | 475       | 22 (4.6)              |
|                                              | Ad libitum/automatic           | 46       | 1,663     | 43 (2.6)              |
|                                              | No information                 | 3        | 185       | 1 (0.5)               |

Continued
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 \( \text{H}_0: \theta = 1 \). The frailty effect equals the strength of association between 2 individuals within the same herd, measured by Kendall’s \( \tau (1 - \theta) \), 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-
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*

### 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 | *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 (7.4)       | 2 (2.9)       | 5 (0.7)              | 31 (4.5)      | 35 (5.0)      |

**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.
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 all dehornings were at increased risk of diarrhea (hazard ratio [HR] = 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     | —            | —   | 1.0 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 —          |         |
|                                                                             | No information | 49       | 2,157     | —            | —   | 1.0 NS         |         |
| 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       | —            | —   | 1.0 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 information | 4        | 101       | —            | —   | 1.0 NS         |         |
|                                                                             | No             | 111      | 4,653     | —            | —   | 1.0 —          |         |
| 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            | 3,189    | 0.63      | 0.15         | 1.9 | (1.4–2.5)     | <0.001  |
|                                                                             | No             | 3,912    | 0.73      | 0.15         | 1.9 | (1.4–2.5)     | <0.001  |
|                                                                             | No information | 4        | 4362      | −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...
enteropathogens and diarrhea in calves

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 record-ings 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.

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