Enteropathogens and risk factors for diarrhea in Norwegian dairy calves

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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 $400\times$ 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*, $100\times$ 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.