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Cryptosporidium parvum infection and associated risk factors in dairy calves in western France

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ABSTRACT

This study was conducted to determine the prevalence and risk factors for *Cryptosporidium* infection in calf neonates on dairy farms in Normandy.

Fecal samples were randomly collected between July 2010 and September 2011 from 968 calves (7–21 days old) on 97 farms. Up to 10 calves were selected and sampled per farm, and feces examined for oocysts by microscopy. *C. parvum* oocyst shedding was scored semi-quantitatively (0–5). A questionnaire about calf-level care and management was completed, and mortality rates were obtained from the French national registration database (BDNI). Bivariable and multivariable analyses of potential risk factors for *C. parvum* oocyst shedding were conducted using generalized estimating equation (GEE) models (family = Binomial).Overall, 402 out of 968 calves (41.5%) were positive for oocysts, and 25.1% of animals had a shedding score >2. Seven of the 97 farms (7%) were negative for oocysts in all fecal samples. At the time of collection, 375 calves (39%) had diarrhea, and its prevalence strongly correlated with the score for *C. parvum* oocyst shedding ($p < 0.0001$). The mortality rate at 90 days was significantly greater for calves with high combined scores of diarrhea and shedding. Factors associated with the shedding of *C. parvum* were the Normande breed (odds ratio = 1.49; 95% confidence interval (CI): 0.93–2.37), dispensing of colostrum using a bucket (odds ratio = 1.37; 95% CI: 1.00–1.89), treatment with halofuginone (odds ratio = 0.46; 95% CI: 0.19–1.15) and feeding with fermented milk (odds ratio = 0.32; 95% CI: 0.17–0.63).

C. parvum is widespread among calves under 21 days old in dairy herds of western France. Shedding of *C. parvum* is associated with a high incidence of diarrhea and increased risk of mortality in young calves. This study identified some associated calf-level factors, although further investigations are necessary to determine appropriate measures that farmers and veterinary practitioners should take to reduce the prevalence of *C. parvum*.

1. Introduction

Cryptosporidium is one of the most common enteropathogens present in calves during their first two weeks of age (Thompson et al., 2007). Young calves mainly shed *Cryptosporidium parvum*, which has a wide host range and is potentially zoonotic. Clinical infection in calves

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is characterized by non-specific diarrhea, dehydration, depression, anorexia and abdominal pain. In most calves, diarrhea starts 3–5 days postinfection and lasts from 4 to 17 days (de Graaf et al., 1999). Oocyst shedding begins at 4 days after birth and peaks at 7–18 days, decreasing after 3 weeks (Nydam et al., 2001; Trotz-Williams et al., 2007). Higher oocyst shedding is frequently seen during episodes of diarrhea (Xiao and Herd, 1994; McCluskey et al., 1995). Despite much endeavor in the development of effective drugs, halofuginone lactate (HL) is the only drug registered in Europe to treat cryptosporidiosis in cattle. HL can reduce, but not completely prevent, oocyst shedding and diarrhea in calves (Joachim et al., 2003), suggesting that additional hygiene measures must be adopted to provide better control of *Cryptosporidium* infections (de Waele et al., 2010). Other enteric viral and bacterial pathogens such as rotaviruses, coronaviruses and *Escherichia coli* can also cause diarrhea in bovine neonates to the same degree of severity as cryptosporidiosis (de Graaf et al., 1999).

Cryptosporidiosis is a multifactorial disease in which many parameters associated with calves, and environment and production practices, may be of significance. Previous studies have indicated that cryptosporidiosis may occur more frequently in dairy calves than in beef calves because the former are born throughout the year and are confined to pens or hutches, which can facilitate a high level of year-round transmission (Olson et al., 2004). Other factors linked to passive immunity of the calf and management of large herds may also be relevant (O'Handley, 2007). Several factors have been identified as significant in increasing the risk of shedding *Cryptosporidium* oocysts by dairy calves. Most studies have focused on factors associated with management of the herd, and attempted to resolve these issues by presenting a questionnaire to farmers (*e.g.*, Garber et al., 1994; Mohammed et al., 1999; Trotz-Williams et al., 2007; Silverlås et al., 2009b). Significant risk factors may differ from survey to survey, indicating that different epidemiological practices may need to be adopted on different farms. Epidemiological data for dairy calf cryptosporidiosis in France is scarce, and there are no risk factor analysis. The first surveys in France underlined the importance of *Cryptosporidium* in neonatal diarrhea and reported that the prevalence of oocyst shedding ranged from 17.9% to 43.4%, depending on the sampling regime (Naciri et al., 1999; Lefay et al., 2000). Molecular characterization of *Cryptosporidium* isolates shed by dairy calves identified the zoonotic *C. parvum* as the dominant species, followed by *C. bovis* and *C. ryanae* (Follet et al., 2011; Rieux et al., 2013).

The aim of this study was to determine the prevalence and risk factors for *Cryptosporidium* infection in bovine neonates in an area of intensive dairy farming in western France.

2. Materials and methods

2.1. Sampling strategy and data collection

The sampling pool consisted of 1676 herds located in the Orne Department of Normandy (France), from which a computer-generated list of 120 farms was randomly selected (Survey Toolbox software). Farms were visited every two weeks during the calving period between July 2010 and September 2011. Ten calves that were 7–21 days old were sampled on each farm during biweekly visits. Calves were randomly selected if there were more than 10 available.

Individual fecal samples were taken directly from the rectum of each calf using sterile plastic gloves and stored at 4 °C before transfer to the laboratory.

At the time of collection, the consistency of each fecal sample and general condition of the calf were scored according to Naciri et al. (1999) in the following manner: for diarrhea, absence (0), mild (1), heavy (2); and for general condition, normal vitality (0), low vitality (1), very low vitality (2), recumbency (3).

Sampling was done in compliance with animal welfare and did not cause distress according to the French Ethics Committee for Animal Experimentation no. 16.

Information on the calf mortality rate was obtained from the French national registration database (BDNI). Mortality was estimated from the number of calves that died before they reached 90 days. Calves that were sold before 90 days were excluded from this calculation.

2.2. Sample analysis

Screening for oocysts was done by staining fecal smears with Ziehl fuchsin and observing at 100× magnification under a phase-contrast microscope according to Heine (1982). The intensity of shedding was evaluated semi-quantitatively according to the average number of oocysts in 10 randomly selected observation fields. Six categories were defined as follows: 0 (absence of oocysts), 1 (<1 oocyst per field), 2 (1–10 oocysts), 3 (11–20 oocysts), 4 (21–30 oocysts) and 5 (>30 oocysts). One person conducted all tests. Considering direct IFAT as the gold standard, Heine staining of fecal smears has a sensitivity of 76.7% and was used because the scoring (1–5) correlates with the number of oocysts in feces (Chartier et al., 2013).

2.3. Questionnaire

A questionnaire was used to collect information about the management of each calf sampled. Questions were designed to gather information about potential predictors of risk for shedding of *C. parvum*. Data were collected in the field during an interview with the farmer or farm manager.

2.4. Statistical analysis

The positive or negative status of each fecal specimen was scored as a dichotomous variable (Heine technique score ≥ 1 *vs* control: score = 0). All factors investigated were categorical variables collected at the individual level as follows: age, sex, breed, period of birth, type of housing in which the calf was kept, ease of birth, care given to the calf, vaccines and drugs dispensed to the calf, maternal vaccination against neonatal diarrhea, length of time calf stayed with the dam after birth, calving area hygiene, type of colostrum/milk fed, and equipment used to feed calves (Table 1).