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Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves

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ABSTRACT

Between January and April 2007, 424 calves under 22 days of age from 108 Dutch dairy herds were sampled to estimate the prevalence of non-normal faeces ('custard-like'—yellowish-coloured with custard consistency or diarrhoea: watery-like faeces) and the shedding of enteropathogens *Escherichia coli* K99 (*E. coli*), Coronavirus, *Cryptosporidium parvum* (*C. parvum*), Rotavirus and *Clostridium perfringens* (*Cl. perfringens*). In addition, information was collected on animal characteristics and herd-management practices. The probability of detecting each one of five enteropathogens given a calf with 'custard-like' faeces or diarrhoea was estimated using Bayes' rule and was based on the predicted probabilities from a multinomial model including each of five enteropathogens as independent variables. In addition, putative risk factors for the presence of each of five enteropathogens were analysed using logistic regression models with random herd effects.

Fifty-seven percent of calves had faeces of normal colour (brownish) and consistency (firm), 23.8% (95%CI: 19.8–28.2%) had 'custard-like' faeces and 19.1% (95%CI: 15.5–23.2%) had diarrhoea. *E. coli* was the least detected enteropathogen (2.6% (95%CI: 1.3–4.6%) of calves, 9% (95%CI: 5–16%) of herds) and *Cl. perfringens* was most detected (54.0% (95%CI: 49.1–58.8%) of calves, 85% (95%CI: 77–91%) of herds). *E. coli* and Coronavirus were detected incidentally in only one or two calves per herd, whereas *C. parvum* and *Cl. perfringens* were frequently detected in up to four calves per herd. For calves with 'custard-like' faeces, the probability of detecting Rotavirus from a calf in its first week of age was 0.31 whereas for a calf in its second week, there was a 0.66 probability of detecting *C. parvum*. The probabilities of detecting *E. coli*, Rotavirus and *C. parvum* in calves with diarrhoea in their first week of age were 0.10, 0.20 and 0.43, respectively. In calves with diarrhoea between 1 and 2 weeks of age, the probability of detecting enteropathogens was 0.43 for *C. parvum*. None of the tested enteropathogens were related to 'custard-like' faeces or diarrhoea in the third week of age.

Putative risk factors for *E. coli*, Coronavirus and *C. parvum* included the presence of peer-calves shedding Coronavirus, *C. parvum* or Rotavirus, respectively. Additionally, managerial risk factors such as non-optimal hygienic housing (for Coronavirus) and the routine use of antibiotics for diarrhoeic calves (for *C. parvum*) were found. No animal or managerial factors were associated with shedding of *Cl. perfringens*.

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1. Introduction

In order to develop into heifers that are optimally prepared for future production, it is beneficial that calves

grow up without disease problems (Quigley et al., 1996). The most important causes of health problems in calves (0–1 years of age) are intestinal and respiratory problems (Waltner-Toews et al., 1986a; Kaneene and Hurd, 1990). Diarrhoea during the first month of life causes disease and mortality or can lead to delayed growth of calves and a higher age at first calving. These complications have severe direct and indirect economic consequences (Tzipori, 1981; de Graaf et al., 1999; Olson et al., 1999).

Diarrhoea in dairy calves has a multifactorial aetiology, in which viruses, bacteria, protozoa, and also management factors (housing, feeding, hygienic conditions) play a role (Bendali et al., 1999; Lorenz, 2006).

With regard to the enteropathogens, *Escherichia coli* K99 (*E. coli*), Coronavirus, *Cryptosporidium parvum* (*C. parvum*) and Rotavirus have been detected in 75–95% of the cases of intestinal infections in young calves (<1 month) (Acres, 1985; Tzipori, 1985). Although these enteropathogens may not always cause diarrhoea, García et al. (2000) showed a strong association between the presence of one of these intestinal agents and diarrhoea. *Clostridium perfringens* (*Cl. perfringens*) is considered as an important human and veterinary enteropathogen because these gram positive, anaerobe bacteria produce endotoxins. *Cl. perfringens* has been reported to play a role in a broad array of enteric and histotoxic infections (Petit et al., 1999; Smedley et al., 2004) and cause enterotoxaemia in both dairy cows and calves (Musken et al., 2007; Lebrun et al., 2007).

A study in diarrhoeic calves in Belgium (de Graaf et al., 1999) estimated the prevalence of *E. coli*, Rotavirus, Coronavirus and *C. parvum* at 4%, 20%, 8% and 31%, respectively. In a recent Swiss study on diarrhoeic calves, the prevalences for these enteropathogens were 6%, 59%, 8% and 55%, respectively (Uhde et al., 2008). Similar prevalences were estimated in Sweden and Switzerland (Björkman et al., 2003; Luginbühl et al., 2005).

For adequate treatment and prevention, it is necessary to know the cause of diarrhoea. Clinical examination is insufficient to differentiate between possible causes of diarrhoea and needs to be supported by laboratory diagnostic techniques (Nussbaum et al., 1999). This is not feasible for all diarrhoeic calves because of financial and logistic limitations. However, veterinary practitioners need information on the prevalence of enteropathogens, on the relative contribution of enteropathogens to non-normal faeces, and on risk factors related to the presence of these enteropathogens. That information is currently not available for Dutch dairy calves.

The aim of this study was therefore to estimate the prevalence of five important enteropathogens in calves of 1–21 days of age from a random set of dairy herds and to relate this with various types of faeces. From this information, the probability of finding any one of these five enteropathogens, given a calf of a certain age with non-normal faeces, was quantified. In addition, calf- or herd-management putative risk factors associated with each enteropathogen were determined.

2. Materials and methods

2.1. Selection and sampling of dairy herds

The sample size was determined for a design prevalence of 50% with a maximum error of 5%. This meant that 400 calves between 1 and 21 days of age were needed. To determine the number of herds for sampling, we estimated the mean number of calves born each month on an average Dutch herd. In 2007, the median herd size was 70 dairy cows (interquartile range 45–90 cows) and assuming a year-round calving pattern, a mean of 6 (range 4–8) calves were born each month. Based on this estimation, we decided to visit a minimum of 70 herds in order to collect at least 400 faecal samples. From a list of all 21,000 Dutch dairy herds, 400 herds were randomly selected and contacted by telephone.

The inclusion criterion was the presence of two or more calves between 1 and 21 days. Herds with only one calf in that age-group were excluded to facilitate the quantification of clustered presence of enteropathogens within herds. A maximum of 6 samples per herd were taken in order to reduce an excessive effect of herds with many calves and to include at least 70 herds. When more than six calves were present, a random selection of calves was sampled equally from the three age categories (1–7 days; 8–14 days; 15–21 days).

Herd visits were conducted by two investigators and followed a fixed protocol by working from young to older calves. All calves were clinically examined and clinical data (rectal temperature, heart beat, respiratory rate, turgor, and colour of mucosal membranes) as well as data on breed, sex, date of birth, treatments given and disease history were recorded per calf. Rectal faecal samples were taken with a new plastic glove from each individual calf. Subsequently, the faecal samples were scored for consistency and collected in a plastic pot. For the evaluation of faecal consistency the following criteria were defined—score of faeces = 0 or 'normal': firm consistency, brown colour, perineum and tail of the calf is clean and dry; score = 1 or 'custard-like': a paste-like consistency, yellow colour and perineum and/or tail of the calf is smeared with faeces; score = 2 or diarrhoea: watery consistency, perineum and/or tail of the calf is smeared with watery faeces. Subsequently, a questionnaire was conducted asking about management practices. Herdsmen were asked about procedures related to housing (group or individual housing of calves, age in days at which calves were transferred to another housing system), on provision of colostrum (time after birth calves were first fed colostrum, quantity (litres) and frequency per day), provision of milk replacer (day of first provision, quantity (litres) per day), provision of roughage (first day of provision), cleaning procedures for boxes (frequency of cleaning) and utensils (cleaning of utensils with cold or hot water, use of soap or detergents), treatment procedures for diarrhoeic calves (preventive use of antibiotics (yes/no), curative use of antibiotics (yes/no), use of oral rehydration salts (ORS) (yes/no)) and nursing procedures (same person taking care of calves all the time (yes/no), use of specific clothing when caring for the calves (yes/no)). In addition, the feeding regime on the day of sampling was asked (milk replacer or bulk milk or

individual cow milk or water or ORS). Before the start of the study, both investigators were simultaneously trained in two herds by a bovine health specialist in order to standardize the use of clinical scores. Additionally, three herds were visited to evaluate the structure and the flow of the questions.

2.2. Laboratory analysis

Faecal samples were tested for the presence of *E. coli* (K99/F5), Rotavirus, bovine Coronavirus and *C. parvum* using a tetrakit assay (Tetraquick, Bio-X Diagnostics, Belgium). This assay is a chromatographic lateral flow immunoassay coated with coloured gold colloidal reagents and labelled with monoclonal antibodies specific for the enteropathogens. Reported sensitivity and specificity for testing *E. coli* are 90% and 98.5% relative to the ELISA BIO K99, for testing Rotavirus are 96% and 100% relative to dsRNA electrophoresis on PAGE, for testing Coronavirus are 88.9% and 98.7% relative to ELISA BIO K068, and for testing *C. parvum* are 94.1% and 95.5% relative to flotation. Presence of *Cl. perfringens* was based on culture on an anaerobic blood agar plate. *Cl. perfringens* was only scored as absent or present and no further typing for toxin production was done.

2.3. Statistical analysis

Testing for differences in herd size between non-participating and participating farmers was done by the Wilcoxin rank-sum test whereas testing for differences between regional distributions was done by proportion test. The apparent animal prevalence of an enteropathogen was estimated as the number of calves in which the enteropathogen was detected divided by the total number of calves sampled. The same method was applied for the estimation of the prevalence of normal faeces, 'custard-like' faeces and diarrhoea. Herd prevalence was estimated as the number of herds with at least one calf in which the enteropathogen was detected divided by the total number of herds.

To study the extent to which an enteropathogen was associated with the appearance of non-normal faecal, a multinomial regression analysis was performed. The outcome variable 'faecal consistency' had three possible variables (normal, 'custard-like' and diarrhoea) with no ordering between these categories was assumed (mlogit, STATA/SE release 10, Statacorp., 2007). The presence of each one of five enteropathogens and all possible two-way interactions were used as independent variables. The probability of a certain enteropathogen, given 'custard-like' or diarrhoea per week of age, was estimated using Bayes' rule. For example, in the case of diarrhoea, the Bayes' rule was written as

$$\text{Prob}(\text{pathogen}/\text{diarrhoea})_i = \frac{\text{Prob}(\text{pathogen})_i}{[\text{Prob}(\text{diarrhoea})_i] \times \text{Prob}(\text{diarrhoea}/\text{pathogen})_i}$$

where $\text{Prob}(\text{pathogen})_i$ is the prevalence of the enteropathogen per week of age, $\text{Prob}(\text{diarrhoea})_i$ is the prevalence of diarrhoea per week of age and $\text{Prob}(\text{diarrhoea}/$

$\text{pathogen})_i$ is estimated using the predicted probability from the multinomial regression for diarrhoea per week of age. $\text{Prob}(\text{pathogen}/\text{diarrhoea})$ was calculated only if $\text{Prob}(\text{diarrhoea}/\text{pathogen})$ was statistically significant in the multinomial model.

The intra-class correlation coefficient (ICC) was calculated to quantify the extent to which a particular enteropathogen was clustered within a herd. The ICC value can vary between '0' (infections in calves of the same herd were not related) and '1' (all calves suffer from the same infection).

To evaluate putative risk factors for the presence of each of the five enteropathogens, a logistic regression analysis was performed including calf-associated factors (age, sex, breed, clinical information and applied medication for diarrhoea) and herd-associated factors on housing, colostrum, milk replacer, roughage, routine treatment of diarrhoeic calves and the presence of enteropathogens in any of the other tested calves. To account for clustering (calves within a herd) a logistic model with random herd effects was used. A backward procedure was used and included calf- and herd-associated factors that yielded *P*-values <0.25 in preceding bivariable analyses. As a indicator of model fit, the pseudo-*R*-squared based on ordinary logistic regression analysis was calculated as *R*-squared calculations for random-effects models were not available in the statistical package used (STATA/SE release 10, Statacorp, 2007).

For all (except bivariable screening) analyses, a *P*-value of <0.05 was considered to be a significant association.

3. Results

In total, 309 randomly selected dairy farmers were approached by telephone. Of these, 38 were not interested and 9 could not be contacted despite several attempts. Another 142 herds had none or only one calf aged between 1 and 21 days and therefore were excluded. In 12 herds, the criteria for age or minimum number of two calves per herd at the time of actual visiting were not met. Thus, 424 calves investigated in 108 herds were used for the analyses with 2, 3, 4, 5 and 6 calves tested in 21, 21, 28, 21 and 17 herds, respectively. There were 173 (41%) calves tested in their first week of age, 153 (36%) in their second week and 98 (23%) in their third week of age. Herd size and regional distribution of the non-participating herds were not statistically different from the participating herds. One of the included herds had an organic management while all other herds were managed conventionally.

3.1. Prevalence of non-normal faeces

The prevalence of normal, 'custard-like' faeces and diarrhoea was 57.1% (95%CI: 52.2–61.8%), 23.8% (95%CI: 19.1–28.2%) and 19.1% (95%CI: 15.5–23.2%), respectively. During the second week of age, the percentage of calves with non-normal (51.6% (95%CI: 43.4–59.8%)) faeces was higher than in the 1st and third week of age (38.2% (95%CI: 30.9–45.8%) and 37.8% (95%CI: 28.2–48.1%), respectively). At the herd-level, 58% (95%CI: 48–68%) of herds had 1–4 calves with 'custard-like' faeces and 50% (95%CI: 40–60%)

Table 1

Apparent animal prevalence (%) of enteropathogens in Dutch dairy calves by age (days) measured with “Tetraquick[®]” (for *E. coli*, Coronavirus, *Cryptosporidium parvum* and Rotavirus) and bacteriological culture (*Clostridium perfringens*). In total 424 calves in 108 herds were sampled between January and April 2007.

Age (days)	<i>E. coli</i>	Corona	<i>C. parvum</i>	Rota	<i>Cl. perfringens</i>
1–7	4.6	2.9	15.6	14.5	64.2
8–14	0.7	4.7	49.7	25.5	51.0
15–21	2.0	1.0	15.3	11.2	40.8
Total (95%CI)	2.6 (1.3–4.6)	3.1 (1.6–5.2)	27.8 (23.6–32.4)	17.7 (14.2–21.7)	54.0 (49.1–58.8)

^a A chromatographic lateral flow immunoassay coated with coloured gold colloidal reagents and labelled with monoclonal antibodies specific for the enteropathogens (Bio-X Diagnostics, Belgium).

of herds had 1–4 calves with diarrhoea. Only 26% (95%CI: 17–34%) of herds had neither calves with ‘custard-like’ faeces nor calves with diarrhoea.

3.2. Prevalence of enteropathogens

The prevalence of enteropathogens in calves varied from low for *E. coli* (2.6% (95%CI: 1.3–4.6%) and Coronavirus 2.8% (95%CI: 1.6–5.2%) to high for *Cl. perfringens* (54.0% (95%CI: 49.1–58.8%)) (Table 1). At the herd-level, *E. coli* (9% (95%CI: 5–16%)) and Coronavirus (12% (95%CI: 7–20%)) were also found in a limited number of herds whereas *Cl. perfringens* was detected in 85% (95%CI: 77–91%) of herds. For *E. coli* and Coronavirus, the infection was limited to only one or two calves in a herd (Table 2). This was different for the other three enteropathogens, which were frequently found in multiple calves per herd.

When combining the test results, only 119 (28.1% (95%CI: 23.8–32.6%)) calves were detected without any enteropathogen, while 25.7% (95%CI: 21.6–30.1%) calves had two or more enteropathogens (Table 3). This was largely

due to *Cl. perfringens*: the percentage of the calves with two or more enteropathogens was 9.9% (95%CI: 7.2–13.2%) when *Cl. perfringens* infections were not taken into account. In this case, the combination of *C. parvum* and Rotavirus was most prevalent ($N = 33$ calves) (data not shown).

3.3. Intra-class correlation coefficient

The extent to which an enteropathogen was present in multiple calves within the same herd was quantified by the ICC (Table 3). The calculation of an ICC for Coronavirus was not applicable as detection was limited to only one calf per herd. For other enteropathogens the ICC varied from 0.19 for Rotavirus to 0.33 for *C. parvum*.

3.4. Relationship between occurrence of non-normal faeces and presence of enteropathogens

To a varying degree, there was a relationship between the enteropathogens cultured and faecal consistency (Table 4). *E. coli*, *Cl. perfringens*, *C. parvum* and Rotavirus

Table 2

Between herd prevalence (%) (95% confidence interval) of enteropathogens in Dutch dairy calves (1–21 days), percentage of herds with 1–6 calves positive for any of the enteropathogens and the intra-class correlation coefficient (ICC). In total 424 calves in 108 herds were sampled between January and April 2007.

	<i>E. coli</i>	Corona	<i>C. parvum</i>	Rota	<i>Cl. perfringens</i>
Herds	9 (5–16)	12 (7–20)	57 (48–67)	46 (37–56)	85 (77–91)
Number of calves per herd					
1	8	12	29	31	26
2	1		14	10	24
3			8	4	16
4			5	2	11
5			2		4
6					5
Intra-class correlation coefficient (95%CI)	0.27 (0.03–0.85) ^a	Not applicable	0.33 (0.19–0.51)	0.24 (0.12–0.40)	0.19 (0.07–0.43)

^a Not statistically significant at P -value = 0.05.

Table 3

Percentage of Dutch dairy calves with none, 1 and 2 or more enteropathogens including and excluding *Clostridium perfringens*. In total 424 calves in 108 herds were sampled between January and April 2007.

No. of intestinal pathogens	Age week 1 (N = 173)	Age week 2 (N = 153)	Age week 3 (N = 98)	Overall	Overall, excluding <i>Cl. perfringens</i>
None	26.0	20.3	43.9	28.1	59.8
1	50.9	41.8	44.9	46.2	30.4
≥2	23.1	37.9	11.3	25.7	9.8

Table 4

Apparent animal prevalence of enteropathogens stratified by three categories of faecal consistency in Dutch dairy calves (1–21 days). In total 424 calves in 108 herds were sampled between January and April 2007.

Faeces consistency	<i>E. coli</i>	Corona	<i>C. parvum</i>	Rota	<i>Cl. perfringens</i>
Normal	1.7	8.3	15.3	12.0	50.8
'Custard-like'	3.0	5.0	29.7	20.8	50.4
Diarrhoea	4.9	7.4	63.0	30.9	67.9

were cultured more frequently in non-normal faeces whereas for Coronavirus this was not true.

Based on the outcomes of the multinomial regression analyses, Rotavirus detection in the first week increased the odds for 'custard-like' faeces compared to normal faeces (OR = 5.8 (95%CI: 1.9–18.0)). The probability of detecting Rotavirus given 'custard-like' faeces in the first week of age was 0.31 (Table 5). *C. parvum* detection in the second week of age increased the odds for 'custard-like' faeces by 2.4 (95%CI: 1.1–5.4) compared to normal faeces and the probability of *C. parvum* detection in 'custard-like' faeces was 0.66. *E. coli*-, *C. parvum*- or Rotavirus detection in the first week of age increased the odds for diarrhoea compared to normal faeces by 8.6 (95%CI: 1.2–63.8), 11.2 (95%CI: 3.8–33.0) and 6.8 (95%CI: 1.9–23.8), respectively, and the probabilities for each of these enteropathogens to be present in diarrhoeic calves in the first week of age were 0.10, 0.43 and 0.20. In the second week of age, detection of *C. parvum* or *Cl. perfringens* increased the odds for diarrhoea by 8.2 (95%CI: 3.2–21.3) and 3.7 (95%CI: 1.5–9.4) and the probability of finding *C. parvum* or *Cl. perfringens* in diarrhoeic calves of this age was 0.43 and 0.29. None of the two-way interactions between enteropathogens were statistically significant in this model.

3.5. Putative risk factors

A putative risk factor for the presence of *E. coli* in faeces of calves between 1 and 21 days was the presence of

Table 5

Relationship between faecal consistency and enteropathogens in Dutch dairy calves per week of age. The probability of detecting 'custard-like' faeces or diarrhoea given one of the five enteropathogens is expressed as odds ratios from a multinomial regression model, whereas the probability of finding one of the five enteropathogens given 'custard-like' faeces or diarrhoea is calculated using Bayes' rule. These probabilities were only calculated for pathogens that had a significant OR in the multinomial regression. In total 424 calves in 108 herds were sampled between January and April 2007.

Week of age	Enteropathogen	'Custard-like' faeces			Diarrhoea			
		Multinomial model		Bayes' rule Probability	Multinomial model		Bayes' rule Probability	
		OR (CI)	P-value		OR (CI)	P-value		
1	<i>E. coli</i>	2.5 (0.3–21)	0.41	0.31	8.6 (1.2–64)	0.04	0.10	
	Corona	6.3 (0.5–77)	0.15		3.0 (0.2–59)	0.47		
	<i>C. parvum</i>	1.3 (0.4–5.0)	0.68		11.3 (3.8–33)	<0.01		0.43
	Rota	5.8 (1.9–18)	<0.01		6.8 (1.9–24)	<0.01		0.20
	<i>Cl. perfringens</i>	1.0 (0.4–2.3)	1.00		0.9 (0.3–2.4)	0.78		
2	Corona	3.3 (0.3–41)	0.34	0.66	4.3 (0.4–46)	0.23	0.43	
	<i>C. parvum</i>	2.4 (1.1–2.4)	0.03		8.2 (3.2–21)	<0.01		
	Rota	1.2 (0.5–3.2)	0.65		1.4 (0.5–3.9)	0.48		
	<i>Cl. perfringens</i>	1.1 (0.5–2.4)	0.16		3.7 (1.5–9.4)	<0.01		0.29
3	<i>C. parvum</i>	3.0 (0.8–11)	0.10		3.7 (0.7–20)	0.12		
	Rota	0.5 (0.1–2.6)	0.39		1.3 (0.2–8.6)	0.76		
	<i>Cl. perfringens</i>	1.1 (0.4–2.8)	0.86		1.5 (0.4–6.1)	0.53		

The R-squared of the multinomial models were 0.15, 0.13 and 0.03 for the first, second and third week of age, respectively.

Coronavirus in any of the sampled calves in the same herd. Its presence increased the odds of *E. coli* 5.3-fold (95%CI: 1.5–18.3).

The presence of *E. coli* or *C. parvum* in any of the sampled calves of the same herd increased the odds of detecting Coronavirus (OR = 6.1 (95%CI: 1.5–25.5) and 8.3 (95%CI: 1.0–69.5)), respectively (Table 6). Consistently cleaning of housing after removal of calves was a protective factor (OR = 0.15 (95%CI: 0.04–0.53)) for the detection of Coronavirus. And calves that had been treated for diarrhoea before sampling but during their lifetime were more likely to be Coronavirus faecal-positive (OR = 5.9 (95%CI: 1.6–22.0)).

The presence of Rotavirus in any of the sampled calves in the same herd increased the odds of detecting *C. parvum* 2.2-fold (95%CI: 1.0–4.7) (Table 6). There was also a relationship with the feeding regime at the time of sampling. Calves that were given oral rehydration salts (ORS) were more likely to be *C. parvum* positive (OR = 5.1 (95%CI: 1.3–19.8)) compared to calves that were given milk in any form. In addition, on farms where diarrhoeic calves were routinely treated with antibiotics (orally or by injection), the odds of *C. parvum* increased 3.2-fold (95%CI: 1.4–7.3). Calves in their second week of age were more likely to test *C. parvum* positive than younger or older calves.

The odds of Rotavirus increased 2.4-fold (95%CI: 1.1–4.9) when one or more diarrhoeic calves were present at the time of sampling (Table 6). As with *C. parvum*, calves in their second week of age were more likely to test Rotavirus positive compared to calves younger or older (up to 21 days).

No animal- or management-related factors associated with the presence of *Cl. perfringens* in faeces were detected.

4. Discussion

We found that non-normal faeces was very common in calves between 1 and 21 days of age. The prevalence of

Table 6

Results of three separate logistic models with random herd effects indicating herd- and calf-associated risk factors for the presence of Coronavirus, *C. parvum* and Rotavirus in Dutch dairy calves' faeces. In total 424 calves in 108 herds were sampled between January and April 2007.

Enteropathogen	Level	Putative risk factors		Frequency %	Odds ratio	95% lower and upper bound	P-value
Coronavirus ^a	Herd	<i>E. coli</i> detected in calves of the same herd	No	90.1	Reference		
			Yes	9.9	6.1	1.5–25.5	0.01
		<i>C. parvum</i> detected in calves of the same herd	No	37.6	Reference		
			Yes	62.4	8.3	1.0–69.5	0.05
		Housing consistently cleaned after removal of calves	No	17.0	Reference		
			Yes	83.0	0.15	0.04–0.53	<0.01
	Calf	Calf was treated for diarrhoea during its lifetime	No	89.4	Reference		
Yes			10.6	5.9	1.6–22.0	0.01	
<i>C. parvum</i> ^b	Herd	Rotavirus detected in calves of the same herd	No	48.1	Reference		
			Yes	51.9	2.2	1.0–4.7	0.04
		Milk supply at moment of sampling	Milk replacer	37.1	Reference		
			Colostrums	13.0	1.5	0.5–5.1	0.49
			Cow milk	29.7	1.4	0.6–3.2	0.46
			Bulk milk	12.5	1.1	0.–3.6	0.90
	Diarrhoea routinely treated with antibiotics	ORS ^c	7.6	5.1	1.3–19.8	0.02	
		No	47.1	Reference			
		Yes	52.9	3.2	1.4–7.3	<0.01	
	Calf	Week of age	Week 1	40.8	0.1	0.05–0.3	<0.01
			Week 2	36.1	Reference		
			Week 3	23.1	0.1	0.05–0.4	<0.01
		Consistency of faeces	Normal	57.1	Reference		
'Custard-like'			23.8	1.9	0.9–3.9	0.09	
	Diarrhoea	19.1	7.3	3.2–16.0	<0.01		
Rotavirus ^d	Herd	Presence of diarrhoeic calves	No	51.9	Reference		
			Yes	48.1	2.4	1.1–4.9	0.02
	Calf	Week of age	Week 1	40.8	0.6	0.3–1.1	0.07
			Week 2	36.1	Reference		
			Week 3	23.1	0.4	0.2–0.9	0.03
	Consistency of faeces	Normal	57.1	Reference			
		'Custard-like'	23.8	1.8	0.9–3.7	0.10	
	Diarrhoea	19.1	2.5	1.2–5.4	0.01		

^a Pseudo-R-squared = 0.24 based on ordinary logistic regression analysis.

^b Pseudo-R-squared = 0.26 based on ordinary logistic regression analysis.

^c Oral rehydration salts.

^d Pseudo-R-squared = 0.18 based on ordinary logistic regression analysis.

'custard-like' faeces was as high as the prevalence of diarrhoea. To our knowledge, no studies have previously estimated the prevalence or incidence of 'custard-like' faeces. Since we used a cross-sectional study design, it was not possible to verify cause–effect relationships. However, we demonstrated a statistically significant relationship between the detection of Rotavirus and *C. parvum* in the faeces of calves and the occurrence of 'custard-like' faeces. Applying Bayes' rule to these results gave a 0.31 probability of detecting Rotavirus in 1-week, and 0.66 probability of detecting *C. parvum* in 2-week-old calves with 'custard-like' faeces. As *C. parvum* and Rotavirus are both considered to be causes of neonatal diarrhoea (Tzipori, 1985; de Graaf et al., 1999; García et al., 2000; Uhde et al., 2008), it could be hypothesized that 'custard-like' faeces might act as an intermediate step in the development of neonatal diarrhoea.

The prevalence of diarrhoea was highest in the second week of age. In a previous Dutch study, the cumulative incidence of diarrhoea in calves between 1 and 21 days was estimated to be 24.0% (Perez et al., 1990) with the peak incidence of diarrhoea being within the first 2 weeks. In a Canadian study, the rate of diarrhoea between birth and

weaning was estimated to be 20% (Waltner-Toews et al., 1986b). Given these and our estimates of diarrhoea, it seems that the occurrence of diarrhoea has remained stable over time. Apparently little progress has been achieved in reducing its occurrence.

We found that *E. coli* and Coronavirus were present in low frequencies and in most situations, infections were limited to one calf per herd. Previous research has indicated a role for *E. coli* (ETEC) in the occurrence of neonatal diarrhoea (Bendali et al., 1999) but little is known about its prevalence. In general, *E. coli* is shed during a short period of time which results in a low prevalence while its incidence and thus impact on calf morbidity and/or mortality might be relatively high. In our study, we did not find a statistical association between *E. coli* prevalence and calf mortality. However, 14 herds (13%) used vaccination against diarrhoea, including protection against *E. coli* and this will have underestimated the *E. coli* prevalence.

E. coli shedding was associated with the presence of Coronavirus in any of the other calves present on farms between 1 and 21 days. This situation applied to 4 out of the 10 herds in which calves with *E. coli* were detected. To our knowledge such a relationship between the simulta-

neous presence of *E. coli* and Coronavirus in calves within the same herd has not been described before.

Other studies investigating Coronavirus in calves have found low prevalences (Björkman et al., 2003; de la Fuente et al., 1998; Uhde et al., 2008). In none of these studies was detection of Coronavirus related to the presence of diarrhoea. We found a similar result in our study: there was no statistical association between the presence of non-normal faeces and Coronavirus. However, we did find a strong relationship between the detection of Coronavirus and previous antimicrobial treatment of calves in the past. A possible explanation might be that Coronavirus is an opportunistic infection that occurs when calves have previously had episodes of diarrhoea and were treated for bacterial infections. Additionally, consistent cleaning of the housing area for calves was a protective factor for calves shedding Coronavirus. This result stresses the importance of good hygienic procedures in calf-rearing facilities.

C. parvum is considered to be an important agent in the aetiology of the neonatal diarrhoeic syndrome of calves and causes considerable direct and indirect economic losses (de Graaf et al., 1999; Naciri et al., 1999; Haschek et al., 2006; Singh et al., 2006). The results of several studies in dairy herds have indicated that *C. parvum* is very common among dairy calves (Garber et al., 1994; de la Fuente et al., 1999; Olson et al., 1999; Ruest et al., 1998; Sischo et al., 2000; Trotz-Williams et al., 2005) with apparent animal prevalences ranging from 15% to 48% for calves between 0 and 3 weeks of age. In these studies, over 60% of herds had one or more infected calves. Trotz-Williams et al. (2008) recently highlighted that most of the variation in shedding occurred between herds, rather than within herds. This indicates that herd-level risk factors, rather than calf-related factors, are important to effectively control *C. parvum* (Trotz-Williams et al., 2008). Our study confirmed this: the ICC for *C. parvum* shedding was as high as 0.33.

In our study, we found that *C. parvum* shedding was increased 3.2-fold on farms that routinely used antimicrobials for treating diarrhoeic calves. This may be an indication that in Dutch dairy herds diarrhoeic calves were treated with antimicrobials correctly, because bacteria were considered the cause of diarrhoea. Our results will be used to motivate herdsmen and veterinarians to include *C. parvum* as a possible cause for diarrhoea and to minimize the routine use of antibiotics. Additionally, in treatment protocols for diarrhoeic calves, submissions of faecal samples for *C. parvum* testing have to be included.

Contrary to a Canadian study (Trotz-Williams et al., 2008), feeding milk replacer was not associated with shedding of *C. parvum* in our study. However, we observed a strong relationship between calves being fed ORS and shedding *C. parvum*. This is consistent with a high probability of detecting *C. parvum* in diarrhoeic calves.

Another risk factor for *C. parvum* was the presence of one or more calves of the same age shedding Rotavirus. Concurrent infections with *C. parvum* and Rotavirus have been described (de la Fuente et al., 1999; García et al., 2000; Björkman et al., 2003; Uhde et al., 2008) and were

confirmed in our study. Together with *C. parvum*, Rotavirus was the infectious agent most frequently found in young calves with enteritis (Snodgrass et al., 1986; Castrucci et al., 1994). Clinical signs are similar to those of cryptosporidiosis and include diarrhoea, depression, anorexia and mild fever. Björkman et al. (2003) found Rotavirus in 24% of calves and concurrent infections with *C. parvum* or *Giardia intestinalis* in 11% of calves. In our study, concurrent infections of *C. parvum* and Rotavirus were found in 33 calves (7.8%), the highest percentage of concurrent infection when excluding co-infections with *Cl. perfringens*. In contrast to other studies, we found a relationship between the occurrence of diarrhoea and shedding of Rotavirus in the first week of age only.

Cl. perfringens was detected in 54.0% of calves and in 85% of dairy herds. Its impact on non-normal faeces in dairy calves remains controversial. We estimated that the probability of detecting *Cl. perfringens* in diarrhoeic calves between 8 and 14 days of age was 29%. This was based on the results of a multinomial model that corrected for the presence of other enteropathogens concurrently. Although *Cl. perfringens* was more often present in two or more calves per herd, no herd-associated risk factors related to its presence were found. There were also no statistical relationships with other enteropathogens. In the literature, there have been contradicting results regarding the relationship between *Cl. perfringens* and the occurrence of neonatal diarrhoea (Deprez et al., 2007; Manteca et al., 2008). Unfortunately, in our study we were unable to differentiate between different types of *Cl. perfringens* and their capability to produce toxins.

Of herds approached but not participating, most had none or only 1 calf present. Our inclusion criterion (herds with two or more calves) may have caused some selection bias as it skewed our sample to larger herds. However, the size of participating herds was not significantly different from herds not participating. Also, the effect of inferring our study results to the Dutch dairy population seems negligible because herd size is increasing rapidly (70 adult cows in 2007 to 79 in 2009, Dutch I&R).

5. Conclusions

Calves between 1 and 21 days of age have non-normal faeces regularly. Particularly, but not only, *C. parvum* is related to diarrhoea in calves up to 14 days of age. Although *Cl. perfringens* was concurrently present with other enteropathogens, its role as an enteropathogen could not be established in this study. Other concurrent infections were seen for *C. parvum* and Rotavirus. For Coronavirus, *C. parvum* and Rotavirus, this study underlined the importance of strict hygiene procedures in calf-rearing and appropriate diagnostic and treatment procedures in diseased calves.

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