Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

The Veterinary Journal 203 (2015) 155–160

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Risk factors for neonatal calf diarrhoea and enteropathogen shedding in New Zealand dairy farms

J. Al Mawly [a], A. Grinberg [b,*], D. Prattley [a], J. Moffat [c], J. Marshall [a], N. French [a]

[a] mEpiLab, Infectious Disease Research Centre, Hopkirk Research Institute, Massey University, Palmerston North, 4410, New Zealand
[b] Infectious Diseases Group, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, 4410, New Zealand
[c] MSD Animal Health, 33 Whakatiki Street, Upper Hutt, Wellington 5018, New Zealand

ARTICLE INFO

Article history:
Accepted 10 January 2015

Keywords:
Calf diarrhea
Risk factors
Rotavirus
Coronavirus
Cryptosporidium parvum
Salmonella

ABSTRACT

To investigate the risk factors for neonatal calf diarrhoea, a cross-sectional study was conducted on 97 New Zealand dairy farms. Faecal specimens from 1283 calves were scored as liquid, semi-solid or solid, and analysed for bovine rotavirus (BRV) and coronavirus (BCV), enterotoxigenic K99+ *Escherichia coli* (K99), *Salmonella* spp. and *Cryptosporidium parvum*. Calf- and farm-level data were collected by means of a questionnaire and the odds of liquid faeces calculated using mixed effects logistic regression models.

Among the infectious agents, only *C. parvum* (odds ratio [OR] = 2.6; 95% confidence interval [CI], 1.3–5.6; $P = 0.02$), BRV (OR = 2.7; 95% CI, 1.3–5.9; $P = 0.01$) and co-infection with more than one agent (compared with mono-infection: OR = 2.5; 95% CI, 1.3–4.8; $P = 0.01$) were associated with increased odds of liquid faeces in calves which were 9 to 21 days old. Housing of calves in open barns so exposing them to the weather was also associated with increased odds of liquid faeces compared with closed barns (OR = 2.1; 95% CI, 1.1–12.2; $P = 0.03$). Vaccinating cows against calf enteropathogens (OR = 0.2; 95% CI, 0.1–0.9; $P = 0.03$), administering waste milk (from mastitis and/or containing antibiotics; OR = 0.4; 95% CI, 0.1–0.8; $P = 0.01$), the sex of calves (females compared to males OR = 0.2, 95% CI, 0.07–0.7; $P < 0.01$), and the use of straw for bedding (OR = 0.2; 95% CI, 0.03–0.9; $P = 0.03$) decreased the odds of liquid faeces. Conversely, in calves that were 1 to 5 days old, only K99 was associated with liquid faeces (OR = 4.6; 95% CI, 1.2–16.1; $P = 0.02$). In this age group, the odds of liquid faeces were smaller on farms where females took care of the calves, compared with males (OR = 0.4; 95% CI, 0.01–0.9; $P = 0.04$).

Introduction

Neonatal calf diarrhoea, defined as diarrhoea manifesting in the first month of life, is a common health and welfare problem on dairy farms worldwide (De la Fuente et al., 1999; Castro-Hermida et al., 2002; Bazeley, 2003). Enterotoxigenic K99+ *Escherichia coli* (K99) and *Salmonella* strains, bovine rotavirus (BRV) and coronavirus (BCV) and the protozoan parasite *Cryptosporidium parvum* are commonly reported endemic microorganisms associated with neonatal calf diarrhoea (Lanz Uhde et al., 2008; Bartels et al., 2010; Izzo et al., 2011). Whereas K99 causes diarrhoea only during the first week of life, BRV, BCV, *C. parvum* and *Salmonella* also affect older calves (Bazeley, 2003; Foster and Smith, 2009; Gulliksen et al., 2009; Izzo et al., 2011).

Conventional wisdom assumes that neonatal calf diarrhoea is determined by complex interplays between the enteropathogens and environmental factors, and it is essential to determine the contribution of each factor so that diagnosis and control strategies can be implemented. Some authors have suggested that the severity of the diarrhoea increases in the presence of co-infections (De la Fuente et al., 1999; Garcıa et al., 2000), and environmental and husbandry practices, such as inadequate colostrum intake, housing types, and poor hygiene, have also been considered risk factors for calf diarrhoea (Waltner-Toews et al., 1986a; Quigley et al., 1995; Bazeley, 2003). Therefore, due to its multifactorial nature, studies of risk factors for neonatal calf diarrhoea should ideally be performed using comprehensive laboratory investigations and multivariable analyses, but data from such studies are scant.

We performed a cross-sectional laboratory and questionnaire-based study of risk factors for neonatal calf diarrhoea on 97 randomly selected New Zealand dairy farms using multivariable analyses. Whereas the primary aim of the study was to assess potential risk factors for diarrhoea, the analysis also evaluated variables associated with enteropathogen shedding.

Materials and methods

Study design and sampling

A cross-sectional faecal sampling was performed during the 2011 calving season from dairy farms located in five North Island (Waikato; Wellington; Northland; Taranaki; Manawatu-Wanganui) and two South Island regions (Canterbury; Southland)

* Corresponding author. Tel.: +64 6 3569099.
E-mail address: a.grinberg@massey.ac.nz (A. Grinberg).

http://dx.doi.org/10.1016/j.tvjl.2015.01.010
1090-0233/

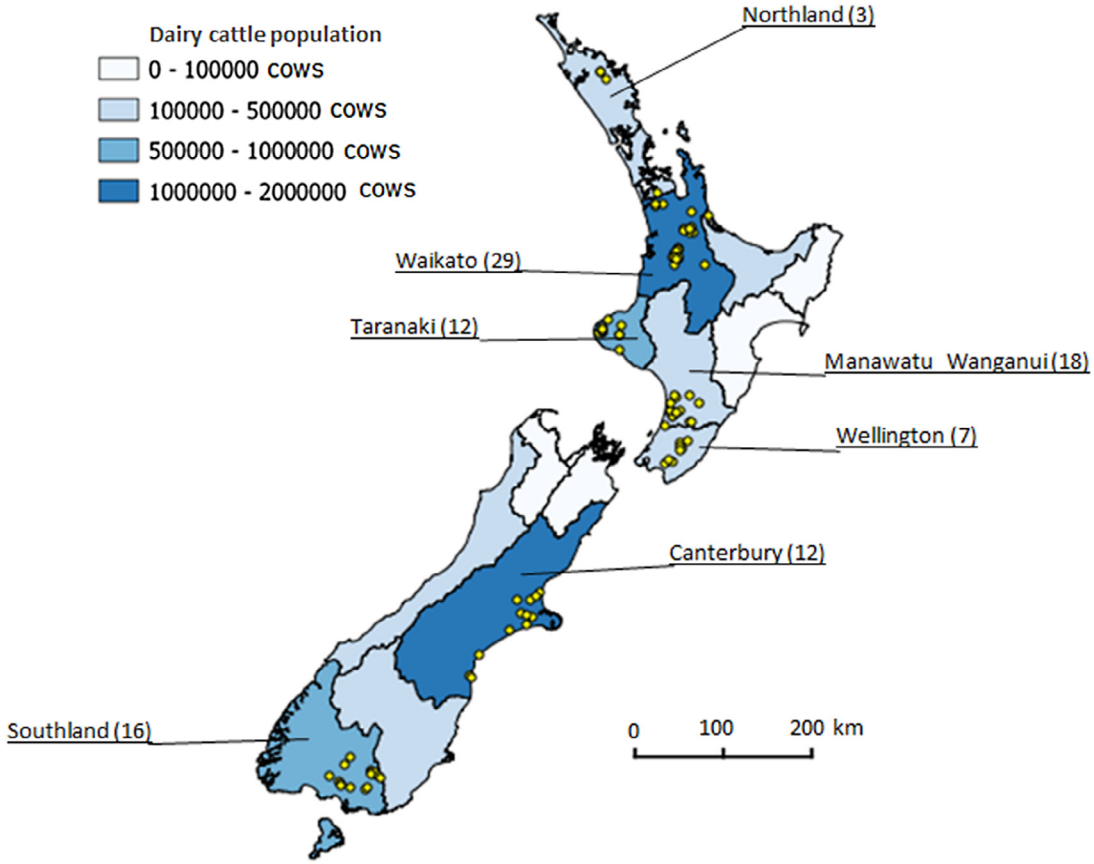

Fig. 1. Spatial distribution of the sampled farms (yellow dots) in the North and South Island of New Zealand. In brackets, the number of sampled farms for each region.

of New Zealand (Fig. 1). Collectively, these regions included ~75% of the national dairy cattle register.[1] The target population was that of all calves on farms milking >150 cows. This minimum farm-size allowed the sampling of multiple calves of the selected ages on each farm, on a single sampling occasion. The sampling frame was represented by all the farms milking >150 cows registered in a database, which included ~10,600 farms milking >150 cows, corresponding to approximately 90% of the estimated number of dairy farms in the country.[2]

The co-ordinates of all the eligible farms were plotted on a New Zealand map delineating regional authorities, and the proportion of farms contributed by each region calculated. A total of 240 farms were selected using random numbers with a regionally proportional sampling scheme. Farmers were contacted by phone and the first 50% willing to participate from each region were recruited. A sample size of 120 was the maximum number that could be reached for sampling during the second half of the calving season. Each farm was visited once. In order to account for the significant differences in the susceptibility of the age-groups to the different infectious agents, two groups of calves were sampled.

The first group was represented by calves which were 1 to 5 days old. This group was targeted to assess the impact of K99, which does not usually affect older calves (Bazeley, 2003; Foster and Smith, 2009). The specimens from these calves were also tested for BRV, BCV, *Salmonella* and C. parvum. The second group was the calves aged 9 to 21 days old, assumed to be at the peak of *C. parvum* shedding (Grinberg et al., 2002). These were tested for BRV, BCV, *C. parvum* and *Salmonella* spp. In a hypothetical calving season of 60 days, after accounting for mortality and culling, a farm milking 150 cows could have presented about five calves aged 1 to 5 days old, and 10 calves aged 9 to 21 days old for sampling.

Samplers collected ~10 g of rectal faeces from calves, changing disposable gloves between animals. The breed, sex and age-group of each animal were registered and a faecal consistency score (1, faeces conserving its shape; 2, faeces spreading across the bottom of the container, but not liquid; 3, liquid faeces) assigned to each specimen. Specimens were analysed for the presence of enteropathogens at Massey University within a week.

[1] See: http://www.asurequality.com/asurequality-global-experts-in-food-safety-and-quality.cfm, accessed February 2011.

[2] New Zealand Dairy statistics 2010-11. DairyNZ. See: http://www.lic.co.nz/pdf/DAIRY%20STATISTICS%2010-11-WEB.pdf, accessed 10 October 2013.

Laboratory analysis

The analyses for enteropathogens have been previously described (Al Mawly et al., 2014). Briefly, BRV, BCV and K99 were tested using a commercial ELISA. *Salmonella* spp. were analysed by culture using two parallel enrichment broths followed by subculture onto differential media. *Cryptosporidium* spp. oocysts were identified using immunofluorescence (IFA). PCR-sequencing of the *Cryptosporidium* 18S rRNA gene was performed to differentiate *C. parvum* from other species. If a *C. parvum* was identified, all the IFA-positive specimens from that farm were considered *C. parvum*-positive.

Collection of farm-level data

Demographic (breed; herd-size), infrastructure (e.g. type of barns, pens, floors, feeders, bedding), and husbandry data (e.g. colostrum and milk feeding practices, hygiene, cows' vaccination against enteropathogens) were elicited by a questionnaire delivered to farmers on the sampling day. Initially, a draft questionnaire was subjected to cognitive evaluation by 15 Massey University students and staff. Questions were modified, a new draft was assessed by three non-enrolled farmers and the final questionnaire prepared.

Data analysis

Data were coded into variables using uniform definitions (Appendix: Supplementary material). Analysis included preliminary explorations, including pairwise analyses for correlation of binary variables using the χ-square test. This was followed by multivariable modelling using mixed effects logistic regression (LogReg). Two main research questions were addressed. These were firstly the risk factors for neonatal calf diarrhoea. The probability of passing liquid faeces at the day of sampling was in theory correlated to the incidence of diarrhoea on the farm, and the duration of the diarrhoea. Thus, this study analysed the presence of variables independently associated with liquid faeces, using the binary outcome: presence/absence of faecal score 3. The second question considered risk factors for enteropathogen shedding, in which we analysed the presence of variables independently associated with the presence of each enteropathogen in faeces using binary outcomes: presence/absence of each enteropathogen (separate univariate models were fitted for each enteropathogen).

Table 1
Number of dairy farms and calves positive for enteropathogens in New Zealand.

Enteropathogen	Number of farms in which the infections were observed	Number of calves positive for these enteropathogens	
		9 to 21-day-old calves	1 to 5-day-old calves
Rotavirus (total)	68/97 (70.1%)	158/797 (19.8%)	86/429 (20%)
Coronavirus (total)	46/97 (47.4%)	49/797 (6.1%)	23/429 (5.3%)
C. parvum (total)	49/97 (50.5%)	126/797 (15.8%)	25/429 (5.8%)
Salmonella spp. (total)	4/97 (4.1%)	4/797 (0.5%)	3/429 (0.6%)
E. coli K99$^+$ (total)	11/97 (11.3%)	Not tested	14/429 (3.2%)
Rotavirus + coronavirus	23/97 (23.7%)	18/797 (2.2%)	10/429 (2.3%)
Rotavirus + *C. parvum*	22/97 (22.6%)	33/797 (4.1%)	9/429 (2%)
C. parvum + coronavirus	6/97 (6.1%)	4/797 (0.5%)	2/429 (0.4%)
E. coli K99$^+$+ rotavirus	8/97 (8.2%)	Not applicable	9/429 (2%)
Rotavirus + coronavirus + *C. parvum*	1/97 (1%)	2/797 (0.2%)	1/429 (0.2%)
E. coli K99$^+$ + *C. parvum*	1/97 (1%)	Not applicable	1/429 (0.2%)
E. coli K99$^+$ + coronavirus	2/97 (2%)	Not applicable	2/429 (0.4%)
E. coli K99$^+$ + *C. parvum* + rotavirus	1/97 (1%)	Not applicable	1/429 (0.2%)
Rotavirus + *C. parvum* + *Salmonella*	1/97 (1%)	1/797 (0.1%)	1/429 (0.2%)

The following LogReg models including fixed effects of explanatory variables of interest were fitted, using the farm identifier as random variable:

$$Y_i = \beta_0 + \beta_1 X_{1i} + \ldots + \beta_k X_{ki} + \mu_{farm(i)} + \varepsilon_i$$

where: Y = outcome variable; β = regression coefficient (β_0 = intercept; $\beta_{1\ldots k}$ = variable coefficients); $X_{1i},\ldots,X_{ki}$ = explanatory variables; $\mu_{farm(i)}$ = farm random effect for the farm of the i_{th} calf; $\mu_{farm(i)} \sim Normal(0, v^2)$; v^2 = variance of random effect of farm; $\varepsilon_i \sim Normal(0, \sigma^2)_{(Residuals)}$ and σ^2 = variance of residuals.

Initially, bivariate screening of each variable against the outcome was performed using the farm identifier as random effect. Variables with at least one comparison with $P < 0.2$ were selected for multivariable LogReg. Biologically correlated variables were not fitted together in models even if the pairwise χ-square test was not significant (see Table 2). Conversely, variables perceived as influential were put together even if the pairwise χ-square test was significant. Separate analyses were performed for each age-group using the 'lme4' and 'Mice' packages on *R* (de Boeck et al., 2011; van Buuren and Groothuis-Oudshoorn, 2011). The imputation function in 'Mice' was used to assign missing sex values before modelling. The function imputed sex values drawn from the binomial probability distribution inferred from the incomplete dataset in each cell containing a missing value, allowing inclusion of these calves. Five randomly selected imputed datasets were retrospectively plotted to verify similar sex distributions in incomplete and imputed datasets. The LogReg results were cross-validated using the dataset containing missing data (without imputation).

Calves with missing age-group were eliminated from the LogReg as the two groups were analysed separately (K99 was not measured in the older group). Also calves from farms containing missing values were eliminated from the LogReg, as imputation of identical values for calves on the same farm was not possible. LogReg was performed by backward variable elimination. Each model was repeated using 50 imputed datasets and the 50 estimates pooled as previously described (van Buuren and Groothuis-Oudshoorn, 2011). The process progressed by elimination of variables with the highest *P*-value, re-introduction of the previously eliminated variable and selection of the model with the smallest Akaike Information Criterion score (Müller et al., 2013). Analyses concluded when only variables with $P < 0.05$ remained.

To control for potential confounding, biologically relevant variables ('vaccination of dams against calf scours' and 'calf's sex') were included in all the models, regardless of their *P*-value. Variability attributable to farm effects was assessed in *R* by visualisation of caterpillar plots. If needed, ad hoc models were used (see explanation for ad hoc models of BCV and co-infection in Results). Odds ratios (OR), 95% confidence intervals (CI) not overlapping the null value and $P < 0.05$ were considered significant. Final model diagnostics included assessment for an abnormally high/low OR and/or wide CI.

Results

Descriptive data analysis

Due to field constraints, 97/120 farms (81% of target) and 1283 calves could be eventually sampled. Samples from 55/1283 calves (4.2%) had no sex recorded and 57/1283 (4.4%) no age-group record. Out of 1226 calves with specified age-group, 797 (65%) were from calves that were 9–21 days old and 429 (35%) from the calves that were 1–5 days old. There were 693 (87%) females that were 9–21 days old and 262 (61%) that were 1–5 days old (this difference was most likely a consequence of culling of males). In total, 116/1226 (9.5%) specimens had faecal score 3 (61/429 from 1 to 5-day-old and 55/797 from 9 to 21-day-old calves). Liquid specimens were identified in 51/97 (52%) farms. Only three farms had missing values at the farm level.

PCR-sequencing revealed *C. parvum* and *C. bovis* and only one species was identified on each IFA-positive farm. *C. parvum* accounted for 92% of the specimens on 89% of the genotyped farms. *Cryptosporidium* species could not be defined by PCR-sequencing on eight farms, and these calves were excluded. The numbers of farms and calves infected with enteropathogens are shown in Table 1. The laboratory results and questionnaires generated 41 variables (supplementary material).

Results of LogReg modelling for calves that were 1 to 5 days old

Bivariate screening identified 12 variables associated at $P < 0.2$ with liquid faeces in this age group (Table 2). Among the enteropathogens, only K99 was positively associated with liquid faeces in this age group (OR = 4.6; 95% CI, 1.2–16.1; $P = 0.02$) (*Salmonella* was found in only three specimens and BCV was not included in multivariable modelling due to $P > 0.2$ in bivariate screening). An ad hoc LogReg model which included only the explanatory variables of presence/absence of the enteropathogens (and the random effect of farm) did not change the significance of these results. In the final model, the odds of liquid faeces was lower where only females (OR = 0.4; 95% CI, 0.1–0.9; $P = 0.04$), or females and males (OR = 0.2; 95% CI, 0.01–0.8; $P = 0.02$) were employed as calves caretakers, compared with farms employing only males (Table 3). Cross-validation without imputation did not change the direction and significance of these results and caterpillar plots did not indicate significant variation between the effects of the farms. No abnormal ORs and CIs were revealed in the final model (Table 3). Finally, no variables associated with increased odds of shedding of the enteropathogens were identified in this age group.

Results of LogReg modelling for calves that were 9 to 21 days old

Bivariate screening identified 15 variables associated at $P < 0.2$ with liquid faeces in this age group (Table 2). Nine variables remained significant in the final model (Table 3). In particular, the presence of *C. parvum* (OR = 2.6; 95% CI, 1.3–5.6; $P = 0.02$) and BRV (OR = 2.7; 95% CI, 1.3–5.9; $P = 0.01$) was independently associated with increased odds of liquid faeces. Conversely, BCV and *Salmonella* were not significant (*Salmonella* was found in four specimens and BCV was not included in multivariable modelling due to $P > 0.2$ in bivariate screening). BCV was found in a lower number of calves than BRV and *C. parvum* (Table 1). Thus, in order to prevent Type II error, the strength of association between the presence of BCV and liquid faeces was assessed as in the younger calves using a parsimonious model that includedonly variables

Table 2
Variables with $P < 0.2$ in bivariate screening analysis, with farm identifier modelled as random effect.

Variable	Categories	*P*-value, odds ratio (95% confidence interval)	
		1 to 5-day-old calves	9 to 21-day-old calves
Calf level variables			
Cryptosporidium parvum shedding	Yes/No	0.06, 2.6 (0.9–7.3)	0.01, 2.5 (1.1–5.5)
Rotavirus shedding	Yes/No	0.2, 1.5 (0.7–3.3)	<0.01, 3.1 (1.4–6.5)
E. coli K99 shedding (1–5 day-old calves only)	Yes/No	0.02, 4.9 (1.2–19.2)	Not tested
Co-infection (any combination of agents)*	Yes/No	0.9, 0.9 (0.3–3.4)	0.12, 2.2 (0.8–5.8)
Calves' sex	Female/male	0.2, 0.6 (0.3–1.2)	0.12, 0.4 (0.15–1.2)
Farm-level variables			
Dam vaccination	Yes/No	0.7, 1.1 (0.4–2.7)	0.08, 0.5 (0.08–1.00)
Feeders cleaned between pens	Yes/No	0.1, 2.5 (0.8–7.7)	0.58, 0.8 (0.42–1.60)
Use of water blaster	Yes/No	0.09, 2.5 (0.8–7.5)	0.11, 2.1 (0.83–5.27)
Feeding calves with waste milk	Yes/No	0.2, 0.5 (0.2–1.2)	0.01, 0.5 (0.39–0.90)
Importation of cows from other farms	Yes/No	0.1, 0.4 (0.1–1.3)	0.19, 0.5 (0.25–1.31)
Numbers of days calves are kept housed from birth	1–30	Reference category	
	31–60	0.1, 0.3 (0.2–1.1)	0.8, 0.7 (0.1–3.2)
	>60	0.2, 0.7 (0.4–2.2)	0.9, 0.3 (0.09–1.5)
	>3	0.9, 1.1 (0.3–3.7)	0.4, 0.8 (0.2–1.6)
Bedding cleaning method	Topped up	Reference category	
	Topped up+ spray disinfection	0.5, 0.7 (0.2–2.1)	0.07, 0.4 (0.1–1.1)
	Complete replacement	0.1, 0.4 (0.09–1.5)	0.09, 0.3 (0.1–1.2)
	Complete replacement and disinfection	0.1, 0.4 (0.1–1.4)	0.01, 0.2 (0.1–0.7)
Type of litter in pens	Straw	Reference category	
	Sawdust	0.6, 1.3 (0.3–4.9)	0.13, 3.5 (0.7–1.8)
	Woodchips	0.9, 1.0 (0.2–3.7)	0.50, 1.8 (0.3–10.8)
	More than one type	0.5, 1.6 (0.3–6.7)	0.87, 1.2 (0.2–9.1)
Type of barn	Closed barn	Reference category	
	Partially open	0.2, 0.5 (0.1–1.5)	0.03, 3.3 (1.1–9.9)
	Open barn	0.02, 9.4 (1.4–60.2)	0.37, 3.2 (0.2–14)
	More than one type	0.8, 0.9 (0.3–2.2)	0.31, 1.8 (0.6–5.6)
Type of milk fed to calves	Fresh milk	Reference category	
	Powdered	0.7, 1.3 (0.1–11)	0.73, 0.6 (0.1–11.6)
	Fresh and powdered milk	0.1, 0.3 (0.06–1.4)	0.11, 0.2 (0.02–1.5)
Time of first colostrum feeding	Within 2 h	Reference category	
	Within 2–6 h	0.2, 3.5 (0.4–24)	0.38, 2.7 (0.3–24)
	After 6 h	0.7, 1.3 (0.1–10)	0.32, 2.9 (0.3–25)
	More than one system	0.1, 4.2 (0.4–38)	0.19, 5.5 (0.5–28)
Type of colostrum	First colostrum	Reference category	
	Stored colostrum	0.5, 2 (0.1–22)	0.33, 2.9 (0.2–31)
	Mixed colostrum	0.5, 1.4 (0.4–4.4)	0.02, 4.1 (1.1–15)
	More than one type	0.6, 1.2 (0.4–3.4)	0.21, 1.9 (0.6–5.9)
Vaccinate all cows or only a subset*	Not vaccinated	Reference category	
	Vaccinate all cows	0.8, 1.1 (0.5–1.9)	0.09, 0.6(0.24–1.1)
	Vaccinate only a subset of cows	0.3, 1.5 (0.8–3.6)	0.22, 0.3(0.05–1.96)
Gender of caretakers	Females	Reference category	
	Males only	0.04, 1.5 (1.00–1.92)	0.5, 1.1 (0.6–1.6)
	Males and females	0.10, 1.3 (.20–1.8)	0.9, 1.6 (0.2–1.8)
Source of drinking water	Town supply	Reference category	
	Bore hole	0.5, 0.1 (0.06–2.9)	0.34, 0.5 (0.1–2.6)
	Rain water	0.6, 2.5 (0.8–23)	0.77, 1.5 (0.2–30)
	Stream	0.1, 1.6 (0.7–9.6)	0.99, 1.0 (0.1–7.6)
	More than one source	0.4, 2.1 (0.5–12.2)	0.74, 1.9 (0.03–17.1)

Asterisks indicate variables not included in multivariable logistic regression models due to possible biologically meaningful collinearity ('Co-infection' depended on variables of presence/absence of the agents and 'Vaccinate all cows or only a subset' depended on 'Dam vaccination').

BCV, BRV, and *C. parvum* (and random effect of farm),with similar results: whereas BRV and *C. parvum* retained their significance, BCV was not significant (not shown).

The results of the preliminary χ-square test indicated that BCV and BRV were correlated, thus each agent was also fitted separately in this model, with consistent results (not shown). The effect of co-infection was analysed by removing the variables presence/absence of *C. parvum*, BRV, BCV and *Salmonella* from the final model and fitting a new variable of values: 1, presence of any mono-infection; 2, presence of any co-infection; 3, absence of infection (and random effect of farm). In this model, the odds of liquid faeces was significantly greater in co-infection compared with the other categories (not shown) (specific co-infection combinations were not assessed due to the large number of permutations needed). Housing calves in open barns where animals were exposed to winter weather significantly increased the odds of liquid faeces compared with closed barns (OR = 2.1; 95% CI, 1.1–12.2; $P = 0.03$).

Administering colostrum within the first 2 h of a calf's life decreased the odds of liquid faeces compared with later administration (OR = 0.4, 95% CI, 0.02–0.8; $P = 0.02$). Conversely, feeding stored (OR = 4.8; 95% CI, 1.1–12; $P = 0.04$), or mixed (OR = 3.3; 95% CI, 1.3–8.8; $P = 0.03$) colostrum increased these odds compared with first-milked colostrum. Interestingly, feeding waste milk (mostly defined by farmers as milk from cows treated with antibiotics and/or mastitic milk) (OR = 0.4; 95% CI, 0.1–0.8; $P = 0.01$), and female calves (OR = 0.2; 95% CI, 0.07–0.7; $P < 0.01$) were associated with lower odds of liquid faeces. The odds of liquid faeces were also lower on farms using straw bedding compared with sawdust (OR = 0.2; 95% CI, 0.03–0.9; $P = 0.03$). Finally, vaccinating cows against calf enteropathogens using combined BRV, BCV and

Table 3
Variables independently associated with liquid faeces with $P < 0.5$ in the final logistic regression models with random farm effect.

Variable description	Outcomes	*P*-value	Odds ratio (95% CI)
9 to 21 day-old calves			
C. parvum infection	No (reference)	0.02	2.6 (1.3–5.6)
	Yes		
Rotavirus infection	No (reference)	0.01	2.7 (1.3–5.9)
	Yes		
Feeding calves with waste milk	No (reference)	0.01	0.4 (0.1–0.8)
	Yes		
Dam's vaccination	No (reference)	0.03	0.2 (0.1–0.9)
	Yes		
Calf's sex	Male (reference)	0.00	0.2 (0.07–0.7)
	Female		
Type of colostrum offered to calves	First colostrum (reference)	0.01 (overall *P*-value)	
	Stored colostrum	0.04	4.8 (1.1–12)
	Mixed colostrum	0.03	3.3 (1.3–8.8)
	More than one type	0.18	1.9 (0.7–5.1)
Timing of first colostrum feeding	6 h from birth (reference)	0.02 (overall *P*-value)	
	Within the first 2 h	0.02	0.4 (0.02-0.8)
	Within 2 to 6 h	0.08	0.3 (0.01–1.2)
	More than one system	0.10	0.6 (0.3–1.7)
Type of barn	Closed barn (reference)	<0.01 (overall *P*-value)	
	Open barn	0.03	2.1 (1.1–12.2)
	Partially open barn	0.04	3.5 (1.1–10.5)
	More than one type of barn	0.35	1.5 (0.1–4.6)
Bedding type	Sawdust (reference)	0.04 (overall *P*-value)	
	Straw	0.03	0.2 (0.03–0.9)
	Woodchips	0.15	0.4 (0.1–1.5)
	More than one type	0.65	0.4 (0.1–1.2)
1 to 5 day-old calves			
E. coli K99 shedding	No (reference)	0.02	4.6 (1.2–16.1)
	Yes		
Caretakers' gender	Males (reference)		
	Females	0.04	0.4 (0.1–0.9)
	Males and females	0.02	0.2 (0.01–0.8)

K99 vaccines decreased the odds of liquid faeces (OR = 0.2; 95% CI, 0.1–0.9; $P = 0.03$).

Cross-validation using the dataset containing missing data did not change these results and caterpillar plots did not indicate variation between the random effects of the farms. No abnormal ORs or CIs were revealed in the final model (Table 3). Finally, no variables associated with increased odds of enteropathogen shedding were observed.

Discussion

We present a cross-sectional risk-factor study of neonatal calf diarrhoea on 97 New Zealand dairy farms milking >150 cows. The analysis of two age-groups allowed accounting for the significant differences which exist in the susceptibility of these age groups to the analysed agents. Potential for some selection bias existed during the recruitment of the farms, as some farmers did not agree to participate. In addition, whereas cross-sectional studies allow sampling of a large number of farms, these studies might not provide cause–effect information when temporal relationships between the variables are unknown. For instance, a single negative laboratory test result for an enteropathogen could have indicated uninfected calves, but also sampling in the pre- or post-patent periods. In the present study, this limitation was in part counterweighted by the significant experimental evidence of the pathogenicity of the analysed agents.

BRV and *C. parvum* were the most common agents and were present in 70% and 50% of the farms, respectively. The prevalence of infected calves was similar in the two age groups for all pathogens, except for *C. parvum*, which was more prevalent in the older calves (Table 1). The results of LogReg indicated a number of infectious and non-infectious factors associated with the presence of liquid faeces, and random effect plots suggested that most factors contributing to the diarrhoea were captured by the final models. Among the infectious agents, only K99 in 1 to 5-day-old and BRV and *C. parvum* in 9 to 21-day-old calves were independently associated with liquid faeces. Interestingly, BCV was not associated with liquid faeces, and this was assessed using parsimonious models, arguing against low statistical power. Although BCV has been considered pathogenic in some countries (Lanz Uhde et al., 2008; Izzo et al., 2011), several studies found no association between BCV and diarrhoea (Björkman et al., 2003; Okur Gumusova et al., 2007; Bartels et al., 2010). Conversely, the lack of association of *Salmonella* with liquid faeces was most likely due to the sporadic occurrence of this bacterium.

In 1 to 5-day-old calves, the presence of BRV and *C. parvum* was not associated with liquid faeces, perhaps reflecting a longer incubation period than K99 (Runnels et al., 1980; Foster and Smith, 2009). This suggests that diagnostic testing for these agents would not predict diarrhoea causation in this age group in New Zealand. Interestingly, our ad hoc model of co-infection provided statistical support to the popular notion that co-infection causes more severe disease than mono-infection.

A number of independent environmental and host-associated risk factors for neonatal calf diarrhoea were identified in this study. In 1 to 5-day-old calves, the negative association between the presence of female caretakers and liquid faeces was consistent with previous reports (Hartman et al., 1974; Losinger and Heinrichs, 1997), suggesting that female workers might provide better neonatal care than males. In 9 to 21-day-old calves, dam vaccination against enteropathogens was associated with decreased odds of liquid faeces, in agreement with the experimental evidence (Gonzalez et al., 2010). Dam vaccination is usually implemented during late pregnancy to increase specific colostral immunoglobulin content. In some studies, vaccinating farms were more likely to manifest diarrhoea than non-vaccinating farms (Waltner-Toews et al., 1986a; Frank and Kaneene, 1993; Bendali et al., 1999). However, the effect of vaccination depends, among other reasons, on the type of circulating agents. Furthermore, in some regions vaccination could be more common in severely affected farms than in farms affected with mild diarrhoea, so comparison of the effect of vaccination between regions is difficult.

In 9 to 21-day-old calves, the odds of liquid faeces were greater in farms using open/partially open barns compared with closed barns. Exposure to winter weather might predispose calves to indigestion and diarrhoea, and provision of shelter could improve calf health. In this age-group, calves on farms reporting colostrum administration within the first 2 h of a calf's life had smaller odds of liquid faeces compared with calves on farms reporting administration within 6 h. Administering first colostrum was also associated with decreased odds of liquid faeces compared with stored or mixed colostrum. These factors might have been acted independently, or through the presence of undetected infectious agents (e.g. calves sampled in pre- or post-patent periods). Nevertheless, delaying colostrum intake is known to decrease intestinal immunoglobulin and fat-soluble vitamin absorption (Bellinzoni et al., 1989; Fayer et al., 2000; Bazeley, 2003), and the results highlight the importance of administering first colostrum as soon as possible after birth.

Interestingly, the odds of liquid faeces were lower on farms using waste milk. According to the responses to the questionnaire, most waste milk originated from cows affected with mastitis or treated with antibiotics (data not shown). Comparable results have been reported previously (Chardavoyne et al., 1979). The potential benefits of the administration of waste milk should, however, be assessed in specifically designed prospective studies,

and the risk for development of antimicrobial resistance should also be taken into account.

The 9 to 21-day old female calves had lower odds of liquid faeces than males. Whereas male calves might have little economic value, the value of replacement females is high, potentially influencing neonatal care. In some systems, the rates of dystocia are greater in males, which might affect passive immunoglobulin transfer due to reduced calf vigour and delayed colostrum ingestion (Bellows et al., 1982; Johanson and Berger, 2003). The use of straw was also associated with decreased odds of liquid faeces compared to sawdust in this age group. This is consistent with other reports suggesting straw as an optimal bedding material (Brenner et al., 2005; Stull and Reynolds, 2008; Mohler et al., 2009). Furthermore, ingested sawdust might disturb gastrointestinal function.

Finally, in agreement with previous observations (Bartels et al., 2010), no associations between management and infrastructure variables and shedding of enteropathogens were observed in this study.

Conclusions

This study identified a number of risk factors that could be addressed by dairy farmers in order to reduce the burden of NCD. In particular, *C. parvum*, BRV, co-infection with more than one agent and housing calves in open barns were associated with increased odds of liquid faeces in 9 to 21-day-old calves. Conversely, vaccinating cows against calf enteropathogens and administering waste milk, the use of straw for bedding, and a female calf, decreased the odds of liquid faeces. In 1 to 5-day-old calves, the presence of K99 and employing only male caretakers increased the odds of liquid faeces.

Conflict of interest statement

J. Moffat is employed by MSD Animal Health New Zealand, which provided partial funding for this work. None of the other authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was approved by Massey University Animal Ethics Committee application MUAEC 11/37. Funding from MSD Animal Health New Zealand is acknowledged. We thank the farmers and samplers, Kevin Lawrence and other staff and students for their help with the questionnaire, and Liz Burrows and Tessy George for assistance in the laboratory.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2015.01.010.

References

Al Mawly, J., Grinberg, A., Prattley, D., Moffat, J., French, N., 2014. Prevalence of endemic enteropathogens of calves in New Zealand dairy farms. New Zealand Veterinary Journal In press. http://dx.doi.org/10.1080/00480169.2014.966168.

Bartels, C.J.M., Holzhauer, M., Jorritsma, R., Swart, W.A.J.M., Lam, T.J.G.M., 2010. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. Preventive Veterinary Medicine 93, 162–169.

Bazeley, K., 2003. Investigation of diarrhoea in the neonatal calf. In Practice 25, 152–159.

Bellinzoni, R.C., Blackhall, J., Baro, N., Auza, N., Mattion, N., Casaro, A., La Torre, J.L., Scodeller, E.A., 1989. Efficacy of an inactivated oil-adjuvanted rotavirus vaccine in the control of calf diarrhoea in beef herds in Argentina. Vaccine 7, 263–268.

Bellows, R.A., Short, R.E., Richardson, G.V., 1982. Effects of sire, age of dam and gestation feed level on dystocia and postpartum reproduction. Journal of Animal Science 55, 18–27.

Bendali, F., Sanaa, M., Bichet, H., Schelcher, F., 1999. Risk factors associated with diarrhoea in newborn calves. Veterinary Research 30, 509–522.

Björkman, C., Svensson, C., Christensson, B., De Verdier, K., 2003. *Cryptosporidium parvum* and *Giardia intestinalis* in calf diarrhoea in Sweden. Acta Veterinaria Scandinavica 44, 145–152.

Brenner, J., Elad, D., Bernstein, M., Dagoni, I., Pálfi, V., Yadin, H., 2005. The detection of an unidentified type of adenovirus in the stools of calves with weak calf syndrome by use of a commercial kit designed for the detection of human adenoviruses. Journal of Veterinary Medicine, Series B 52, 98–101.

Castro-Hermida, J.A., González-Losada, Y.A., Ares-Mazás, E., 2002. Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). Veterinary Parasitology 106, 1–10.

Chardavoyne, J.R., Ibeawuchi, J.A., Kesler, E.M., Borland, K.M., 1979. Waste milk from antibiotic treated cows as feed for young calves. Journal of Dairy Science 62, 1285–1289.

de Boeck, P., Bakker, M., Zwitser, R., Nivard, M., Hofman, A., Tuerlinckx, F., Partchev, I., 2011. The estimation of item response models with the lmer function from the lme4 package in R. Journal of Statistical Software 39, 1–28.

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

Fayer, R., Morgan, U., Upton, S.J., 2000. Epidemiology of *Cryptosporidium*: Transmission, detection and identification. International Journal for Parasitology 30, 1305–1322.

Foster, D.M., Smith, G.W., 2009. Pathophysiology of diarrhea in calves. Veterinary Clinics of North America – Food Animal Practice 25, 13–36.

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

Gonzalez, D.D., Mozgovoj, M.V., Bellido, D., Rodriguez, D.V., Fernandez, F.M., Wigdorovitz, A., Parreño, V.G., Dus Santos, M.J., 2010. Evaluation of a bovine rotavirus VP6 vaccine efficacy in the calf model of infection and disease. Veterinary Immunology and Immunopathology 137, 155–160.

Grinberg, A., Lopez-Villalobos, N., Markovics, A., Kosak, A., Galindez, J., Tranquillo, V.M., 2002. Controlling the onset of natural cryptosporidiosis in calves with paromomycin sulphate. Veterinary Record 151, 606–608.

Gulliksen, S.M., Jor, E., Lie, K.I., Hamnes, I.S., Løken, T., Åkerstedt, J., Østerås, O., 2009. Enteropathogens and risk factors for diarrhea in Norwegian dairy calves. Journal of Dairy Science 92, 5057–5066.

Hartman, D.A., Everett, R.W., Slack, S.T., Warner, R.G., 1974. Calf mortality. Journal of Dairy Science 57, 576–578.

Izzo, M.M., Kirkland, P.D., Mohler, V.L., Perkins, N.R., Gunn, A.A., House, J.K., 2011. Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. Australian Veterinary Journal 89, 167–173.

Johanson, J.M., Berger, P.J., 2003. Birth weight as a predictor of calving ease and perinatal mortality in Holstein cattle. Journal of Dairy Science 86, 3745–3755.

Lanz Uhde, F., Kaufmann, T., Sager, H., Albini, S., Zanoni, R., Schelung, E., Meylan, M., 2008. Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. Veterinary Record 163, 362–366.

Losinger, W.C., Heinrichs, A.J., 1997. Management practices associated with high mortality among preweaned dairy heifers. Journal of Dairy Research 64, 1–11.

Mohler, M., Izzo, M.M., House, J.K., 2009. *Salmonella* in calves. Veterinary Clinics of North America – Food Animal Practice 25, 37–54.

Müller, S., Scealy, J.L., Welsh, A.H., 2013. Model selection in linear mixed models. Statistical Science 28, 135–167.

Okur Gumusova, S., Yazici, Z., Albayrak, H., Meral, Y., 2007. Rotavirus and coronavirus prevalence in healthy calves and calves with diarrhoea. Medycyna Weterynaryjna 63, 62–64.

Quigley, J.D., Martin, K.R., Bemis, D.A., Potgieter, L.N.D., Reinemeyer, C.R., Rohrbach, B.W., Dowlen, H.H., Lamar, K.C., 1995. Effects of housing and colostrum feeding on serum immunoglobulins, growth, and fecal scores of Jersey calves. Journal of Dairy Science 78, 893–901.

Runnels, P.L., Moon, H.W., Schneider, R.A., 1980. Development of resistance with host age to adhesion of K99+ *Escherichia coli* to isolated intestinal epithelial cells. Infection and Immunity 28, 298–300.

Stull, C., Reynolds, J., 2008. Calf welfare. Veterinary Clinics of North America – Food Animal Practice 24, 191–203.

van Buuren, S., Groothuis-Oudshoorn, K., 2011. Mice: Multivariate imputation by chained equations in R. Journal of Statistical Software 45, 1–67.

Waltner-Toews, D., Martin, S.W., Meek, A.H., 1986a. Dairy calf management, morbidity and mortality in Ontario Holstein herds. III. Association of management with morbidity. Preventive Veterinary Medicine 4, 137–158.