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

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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) decreased the odds of liquid faeces. Conversely, in calves compared with males (OR = 0.4; 95% CI, 0.01–0.9;  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), 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).

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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.\(^1\)

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 \(spp.\) 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.

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 \(\chi\)-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).

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1 See: [http://www.asurequality.com/asurequality-global-experts-in-food-safety-and-quality.cfm](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%202010-11-WEB.pdf](http://www.lic.co.nz/pdf/DAIRY%20STATISTICS%202010-11-WEB.pdf), accessed 10 October 2013.
The following LogReg models including fixed effects of explanatory variables of interest were fitted, using the farm identifier as random variable:

\[ Y = \beta_0 + \beta_1 X_1 + \ldots + \beta_k X_k + \mu_{\text{farm}} + \epsilon_i \]

where: \(Y\) = outcome variable; \(\beta\) = regression coefficient; \(\mu_{\text{farm}} = \text{farm random effect for the } i\text{th farm; } \epsilon_i = \text{residual for the } i\text{th farm}; X_i = \text{explanatory variable}_i; \mu_{\text{farm}} = \text{farm random effect for the } i\text{th farm; } \epsilon_i = \text{residual for the } i\text{th farm.}

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 \(\chi^2\) square test was not significant (see Table 2). Conversely, variables perceived as influential were put together even if the pairwise \(\chi^2\) square test was significant. Separate analyses were performed for each age-group using the ‘im4’ 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 sex values before modelling. The function imputed sex values drawn from the binomial probability distribution inferred from the incomplete dataset in each cell containing sex values before modelling.

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 BCV (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 accounted for 92% of the specimens on 89% of the genotyped farms. Cryptosporidium species could not be defined by PCR-screening on eight farms, and these calves were excluded in Table 2. The laboratory results and questionnaires generated 41 variables (supplementary material).

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 farms that were 1 to 5 days old and 429 (35%) from the calves that were 9 to 21 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-screening revealed C. parvum and C. bovis and only one species was identified on each IFAs-positive farm. C. parvum accounted for 92% of the specimens on 89% of the genotyped farms. Cryptosporidium species could not be defined by PCR-screening on eight farms, and these calves were excluded in Table 2. The laboratory results and questionnaires generated 41 variables (supplementary material).

Table 1

| Enteropathogen         | Number of farms in which the infections were observed | Number of calves positive for these enteropathogens |
|------------------------|------------------------------------------------------|--------------------------------------------------|
| Rotavirus (total)      | 68/97 (70.1%)                                        | 158/797 (19.8%)                                   |
| Coronavirus (total)    | 46/97 (47.4%)                                        | 49/797 (6.1%)                                     |
| C. parvum (total)      | 49/97 (50.5%)                                        | 126/797 (15.8%)                                   |
| Salmonelle spp. (total)| 4/97 (4.1%)                                          | 4/797 (0.5%)                                      |
| E. coli K99* (total)   | 11/97 (11.3%)                                        | Not tested                                       |
| Rotavirus + coronavirus| 23/97 (23.7%)                                        | 18/797 (2.2%)                                     |
| Rotavirus + C. parvum  | 22/97 (22.6%)                                        | 33/797 (4.1%)                                     |
| C. parvum + coronavirus| 6/97 (6.1%)                                          | 4/797 (0.5%)                                     |
| E. coli K99* + rotavirus| 8/97 (8.2%)                                          | Not applicable                                   |
| Rotavirus + coronavirus + C. parvum | 1/97 (1%) | 2/797 (0.2%) |
| E. coli K99* + C. parvum | 1/97 (1%) | Not applicable |
| E. coli K99* + coronavirus | 2/97 (2%) | Not applicable |
| E. coli K99* + C. parvum + rotavirus | 1/97 (1%) | Not applicable |
| Rotavirus + C. parvum + Salmonella | 1/97 (1%) | 1/797 (0.1%) |

Table 2

| Enteropathogen | 9 to 21-day-old calves | 1 to 5-day-old calves |
|----------------|------------------------|-----------------------|
| Salmonella spp. | 4/97 (0.5%)            | 3/429 (0.6%)          |
| E. coli K99*    | 11/97 (11.3%)          | 12/797 (1.5%)         |
| Rotavirus       | 23/97 (23.7%)          | 21/797 (2.7%)         |
| Rotavirus + C. parvum | 22/97 (22.6%) | 29/797 (3.6%) |
| C. parvum + coronavirus | 6/97 (6.1%) | 11/797 (1.4%) |
| E. coli K99* + rotavirus | 8/97 (8.2%) | 12/797 (1.5%) |
| Rotavirus + coronavirus + C. parvum | 1/97 (1%) | 2/797 (0.2%) |
| E. coli K99* + C. parvum | 1/97 (1%) | Not applicable |
| E. coli K99* + coronavirus | 2/97 (2%) | Not applicable |
| E. coli K99* + C. parvum + rotavirus | 1/97 (1%) | Not applicable |
| Rotavirus + C. parvum + Salmonella | 1/97 (1%) | 1/797 (0.1%) |
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 $\chi^2$-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 the agents and ‘Vaccinate all cows or only a subset’ depended on ‘Dam vaccination’.

$\text{C. parvum}$ retained their significance, $\text{Salmonella}$ 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 fresh and powdered 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 2** Variables with $P<0.2$ in bivariate screening analysis, with farm identifier modelled as random effect.

| Variable | Categories | $P$-value | 1 to 5-day-old calves | 9 to 21-day-old calves |
|----------|------------|-----------|------------------------|------------------------|
| Calf level variables | | | 0.06, 2.6 (0.9–7.3) | 0.01, 2.5 (1.1–5.5) |
| Cryptosporidium parvum shedding | Yes/No | | | |
| Rotavirus shedding | Yes/No | | 0.2, 1.5 (0.7–3.3) | <0.1, 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 (0.15–12) |
| Farm-level variables | | | | |
| Dam vaccination | Yes/No | | 0.7, 1.1 (0.4–2.7) | 0.08, 0.5 (0.08–1.00) |
| Feeder cleaned between pens | Yes/No | | 0.6, 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 | Reference category | | | |
| 1–30 | | | 0.1, 0.3 (0.2–1.1) | 0.8, 0.7 (0.1–3.2) |
| >30 | | | 0.2, 0.7 (0.4–2.2) | 0.9, 0.3 (0.09–1.5) |
| Bedding cleaning method | Reference category | | | |
| Topped up | | | 0.9, 1.1 (0.3–3.7) | 0.4, 0.8 (0.16–16) |
| Topped up spray disinfection | | | 1.3, 3.5 (0.7–18) | |
| Complete replacement | | | 0.5, 1.6 (0.3–6.7) | 0.8, 1.2 (0.2–9.1) |
| Type of litter in pens | Reference category | | | |
| Straw | | | 0.1, 0.4 (0.1–1.4) | 0.01, 0.2 (0.07–1.0) |
| Sawdust | | | 0.1, 0.4 (0.1–1.4) | 0.01, 0.2 (0.07–1.0) |
| Woodchips | | | 0.1, 0.4 (0.1–1.4) | 0.01, 0.2 (0.07–1.0) |
| Type of barn | Reference category | | | |
| Closed barn | | | 0.1, 0.4 (0.1–1.4) | 0.01, 0.2 (0.07–1.0) |
| Partially open | | | 0.1, 0.4 (0.1–1.4) | 0.01, 0.2 (0.07–1.0) |
| Open barn | | | 0.1, 0.4 (0.1–1.4) | 0.01, 0.2 (0.07–1.0) |
| More than one type | | | 0.1, 0.4 (0.1–1.4) | 0.01, 0.2 (0.07–1.0) |
| Type of milk fed to calves | Reference category | | | |
| Fresh milk | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Powdered | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Type of colostrum feeding | Reference category | | | |
| Within 2 h | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Time of first colostrum feeding | Reference category | | | |
| Within 2–6 h | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| After 6 h | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Type of colostrum | Reference category | | | |
| First colostrum | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Stored colostrum | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Mixed colostrum | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Vaccinate all cows or only a subset* | Reference category | | | |
| Not vaccinated | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Vaccinate all cows | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Vaccinate only a subset of cows | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Gender of caretakers | Reference category | | | |
| Females | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Males only | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Males and females | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Source of drinking water | Reference category | | | |
| Town supply | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Bore hole | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Rain water | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| Stream | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |
| More than one source | | | 0.1, 0.3 (0.06–1.4) | 0.11, 0.2 (0.02–1.5) |

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’).
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)      |
| Rotavirus infection                   | Yes                       | 0.01    | 2.7 (1.3–5.9)      |
| Feeding calves with waste milk        | No (reference)            | 0.01    | 0.4 (0.1–0.8)      |
| Dam’s vaccination                     | Yes                       | 0.03    | 0.2 (0.1–0.9)      |
| Calf’s sex                            | Male (reference)          | 0.00    | 0.2 (0.07–0.7)     |
| Type of colostrum offered to calves  | First colostrum (reference)| 0.01    | 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    | 0.4 (0.02–0.8)     |
|                                      | 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 (<0.01 overall)| 0.02    | 2.1 (11.2)         |
|                                      | Open barn                  | 0.04    | 3.5 (11.0–10.1)    |
|                                      | More than one type of barn | 0.35    | 1.5 (10.1–4.6)     |
| Bedding type                          | Sawdust (reference)       | 0.04    | 4.6 (1.2–16.1)     |
|                                      | 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 (12.1)         |
|                                      | Yes                       | 0.04    | 0.4 (0.1–0.9)      |
| Caretakers’ gender                    | 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 counterweighed 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 1st 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 acting 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.

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Appendix: Supplementary material

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

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