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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; $P = 0.02$), 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)

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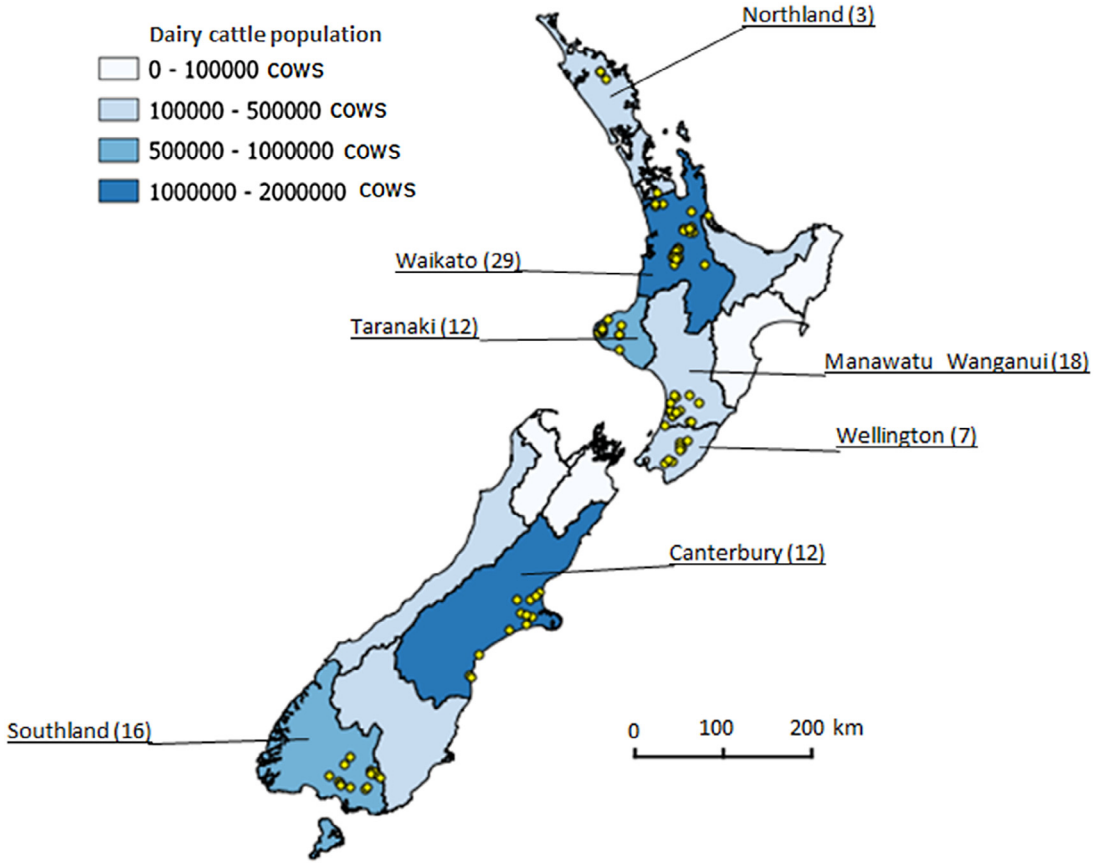

Fig. 1. Spatial distribution of the sampled farms (yellow dots) in the North and South Island of New Zealand. In brackets, the number of sampled farms for each region.

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).