Risk factors for pre-weaning calf morbidity and mortality due to farmer-diagnosed diarrhoea on 45 New Zealand dairy farms

A dissertation presented in partial fulfilment of the requirements for the degree of

Master of Veterinary Studies (Epidemiology)

at Massey University Manawatu

Katharine S. Denholm

2012

Abstract

Aim: To provide an estimate of incidence and identify risk factors for farm-level pre-weaning calf morbidity and mortality due to diarrhoea and describe treatment(s) for pre-weaning calf diarrhoea currently used by herd owners in pasture-based spring management systems in the Waikato region of New Zealand.

Methods: 45 Waikato dairy farmers provided information via a questionnaire about their calf rearing practices. They also provided data allowing the calculation of morbidity and mortality risks of replacement calves due to diarrhoea during 8-12 weeks until weaning. Treatments and deaths were recorded prospectively from 1 July to 30 September 2010. Associations between management factors and the outcomes of farm-level occurrence of one or more cases of calf diarrhoea and farm-level calf morbidity and mortality risk due to diarrhoea were examined using bivariate and multivariable analysis.

Results: Calf diarrhoea was reported from 84% of the 45 dairy farms (3,835 replacements and 13,343 bobby calves). The incidence risk of calf diarrhoea of replacement calves from birth to weaning was 10.0% (95% confidence interval (CI=6.3%-13.6%) and the corresponding mortality risk was 1.0% (CI=0.5%-1.6%), with a case fatality risk of 11.9% (CI=4.6%-19.2%).

The morbidity risk for bobby calves from birth to sale at 5-14 days of age was 1.1% (CI=0.5%-1.7%). The corresponding mortality risk for bobby calves was 0.3% (CI=0.1%-0.5%), with a case fatality risk of 24.0% (CI=9.1%-38.9%).

An increased morbidity risk in replacement calves was significantly ($p<0.05$) associated with the isolation of calves with diarrhoea in a different shed (OR=3.3, CI=1.4-7.4) and feeding of sick calves last (OR=2.8, CI=1.2-6.2). Morbidity risk was lower when hygiene measures such as the use of gloves, footbaths/washing for boots, or both were applied in rearing sheds (OR=0.2, 0.6 and 0.7, CIs=0.07-0.6, 0.3-1.4 and 0.3-1.8, respectively), when calves were first fed once a day at 3 weeks of age (OR=0.5, CI=0.3-1), and when the number of dummy teats per calf was increased or decreased at an older age (OR=0.5, CI=0.2-0.9). Morbidity also decreased with increasing herd size (OR=0.72 per 100 cows, CI=0.6-0.9) or when herds were vaccinated with rotavirus and/or salmonella vaccines (OR=0.4, CI=0.2-0.9).

The odds of replacement calf mortality were lower at increasing age (more than 3 weeks) at which calves were changed to once a day feeding (OR=0.2, CI=0.07-0.7), when water was offered in calf sheds (OR=0.2, CI=0.08-0.7) and when bedding was replaced in calf sheds (OR=0.15, CI=0.07-0.4).

Bobby calf morbidity tended to be higher on farms where disinfecting navel spray was used (OR=2.6; CI=0.9-7.9), when sick calves were isolated in a separate shed (OR=2.2; CI=0.9-5.1) and as more persons were involved in calf rearing (OR=2.5 and 3.6; CIs=0.8-8.3 and 1.6-8.3 respectively). The odds of observed bobby calf morbidity due to diarrhoea was lower in larger herds (OR=0.62 per 100 cows; CI=0.46-0.83).

Changing water in the calf sheds regularly (OR=0.2 to 0.5, CIs=0.07-0.5 and 0.2-1.1 respectively), rearing bobby calves separately from replacement calves (OR=0.1, CI=0.03-0.5) and feeding sick calves last (OR=0.3, CI=0.2-0.6) all reduced the odds of bobby calf mortality significantly ($p<0.01$).

Of the farmers who reported diarrhoeic calves, 82% used electrolyte therapy and 58% used antibiotics, either alone or in combination with electrolytes. Of the farmers who used antibiotics, 35% used oral, while 65% used injectable antibiotics.

Conclusions: The morbidity and mortality risk of pre-weaning replacement calf diarrhoea was quantified for the first time in New Zealand. Both risks were found to be 10-20% lower than risks reported from Canada, Scandinavia and the Netherlands, and 3-7% higher than in Minnesota (USA) and Sweden respectively. However, not all reports from other countries

were comparable to our study due to fundamental differences in calf rearing practices in New Zealand. Considering the 8-9 fold shorter risk period, morbidity and mortality were similar in bobby and replacement calves.

Early feeding once a day, changing dummy teats, dam vaccination pre-calving and the use of gloves and footbaths were all associated with a reduced risk of pre-weaning diarrhoea in replacement calves. Having more than one person in charge of calf rearing increased the incidence of diarrhoea in bobby calves. Offering fresh water and bedding replacement in the shed reduced the mortality risk among replacement calves. Feeding sick calves last, separating bobby from replacement calves and fresh regular water supply were positively associated with bobby calf survival.

Acknowledgements

This study was co-financed by Cognosco research group, DairyNZ and Intervet/Schering Plough. The author would like to thank the participating farmers for their interest and support. Thanks to Cord Heuer at the EpiCentre, Massey University, Palmerston North for his input, to Winston Mason for his help and to Jaimie Hunnam for her patience.

Table of Contents

Introduction

Pre-weaning calf diarrhoea (from birth to weaning) causes significant morbidity and mortality on many New Zealand dairy farms (Vermunt *et al*. 1995). However, farm-level and calf-level recording of calf diarrhoea events and data collection is currently poor, and estimates of the national incidence of pre-weaning calf diarrhoea are not available. Costs of calf diarrhoea in New Zealand have not been estimated. Multiple pathogens of bacterial, viral and parasitic origin may interplay to cause diarrhoeic disease and the multifactorial nature of the disease may make prevention and control challenging.

Most New Zealand dairy herds are seasonal, with calving in spring (LIC New Zealand Dairy Statistics 2010/11). In a proportion of dairy herds, dams are vaccinated against rotavirus and salmonella in the last trimester of pregnancy to increase maternal antibody concentrations, colostral antibody concentrations and hence increase the probability that calves are provided with perinatal immunity (Crouch *et al.* 2001; Eschrig *et al*. 2004; Heckert *et al*. 2005).

Young calves in New Zealand are typically housed in groups and fed on communal gate rail feeders until they are turned out to grass. Calves are typically placed on pasture pre-weaning and fed on 'calfeterias', consisting of large mobile feeders on which multiple teats are mounted to provide suckling points for multiple calves. Calves in New Zealand are often (but not always) fed twice daily while they are very young and then changed in once a day feeding.

Peculiarities of the New Zealand seasonal calving system include selling bobby calves from 4 days of age and weaning of spring born replacement calves at various times depending on weather and other management factors.

Bobby calves are managed differently from replacement calves in that they are kept in a separate pen for ease of pick up. Bobby calves are less valuable (average prices for bobby calves courtesy of industry figures 2010 season $14), and farmer spending on therapy reflects this. They are not fed antibiotic milk and cannot be treated with antibiotics. Bobby calves accidentally fed antibiotic milk must be fed 'clean milk' for at least seven days before they can be sent for slaughter. If a cow is treated with antibiotics during gestation and the calf is born within the withholding period of the drug, then the calf cannot be sent as a bobby calf until after the withholding period is complete. Hence, bobbies are usually sent at four days old, but may remain on farm for longer in some instances. Bobby calf management and removal from the farm at four days of age may affect the pattern of calf diarrhoea and the pathogens responsible for calf diarrhoea in this group of animals.

Replacement stock are usually born in the first month of the calving season, since farmers typically undertake artificial insemination for one month and then allow the bulls to mate the remaining cows. Calves born from bull matings are seldom kept as replacement animals.

Data on weaning age of replacement calves in New Zealand is limited. Starter ration intake is the best method to determine the time of weaning rather than age or liveweight (Schouten 2003). Calves can be weaned when they are consuming 1-1.5kg/day of starter ration (Vermunt 2002). Weaning on the presence of a 'pear-shaped' abdomen (indicating rumen development), instead of weaning on weight or age is recommended (Schouten 2003). Anecdotally speaking, weaning age varies from farm to farm between 6 and 12 weeks of age in New Zealand spring calving dairy herds.

Risk factors for calf diarrhoea have been examined in numerous international studies (Bendali *et al*. 1999; Svensson *et al*. 2003; Bazeley *et al.* 2003). However, the seasonal calving and management systems commonly used in the New Zealand dairy industry may result in different risk factors being significantly associated with pre-weaning calf diarrhoea relative to those identified in previous international studies. Typically, known risk factors for diarrhoea in pre-weaning dairy calves increase infectious pressure or are to the detriment of calf immunity. The placenta of the cow separates the maternal and foetal blood supplies, preventing *in utero* transmission of protective immunoglobulins (Ig). Due to the placentation of ruminants, arguably the most important of the risk factors relate to adequate colostral transfer to the calf (Jaster 2005). The oral intake of antibody-rich colostrum within 24 hours of birth is essential to provide adequate immunity to the calf (McGuire *et al.* 1976; Bush and Staley 1980; Matte *et al.* 1982). A prolonged interval between calving and first milk intake is problematic since calves absorb maternal antibodies through a permeable small intestinal wall for up to 12 hours after birth with the tract becoming less permeable to large immunoglobulin molecules after this time (McGuire *et al*. 1976; Bush and Staley 1980; Matte *et al.* 1982; Jaster 2005). Failure of this colostral transfer results in increased risk of disease. There is much variation in colostral acquired immunity between farms (Anderson 1983; Vermunt 2002). Most new-born dairy calves in New Zealand are fed from pooled colostrum collected in drums and it is often difficult to assess the amount of colostrum that has been ingested by an individual calf. Farmers which tube feed their calves with 2 litres of warm colostrum in the first hours of life may be more likely to achieve more consistent colostral immunity.

The current study was undertaken to:

- Provide an estimate of morbidity and mortality risk of pre-weaning diarrhoea in bobby and replacement calves
- Identify risk factors for morbidity and mortality due to pre-weaning calf diarrhoea
- Describe remedies and administration routes of treatments currently used by herd owners to treat calf diarrhoea.

The outcome of interest was farmer-diagnosed diarrhoea in dairy calves from birth to weaning of undefined aetiology.

Chapter One: Literature Review

Risk Factors for Pre-weaning Calf Diarrhoea in New Zealand Dairy Calves

Diarrhoea is the most commonly reported calf disease and a major cause of calf morbidity and mortality worldwide (Gitau *et al*. 1994; Bendali *et al.* 1999; Bazeley 2003; Svensson *et al*. 2006; Millemann 2009; Marce *et al.* 2010). The average within-herd incidence of diarrhoea in pre-weaning calves is around 20%, varying between 0 and 70% worldwide (Bendali *et al.* 1999). The economic implications of calf diarrhoea include calf losses, treatment costs, time costs and reduced liveweight gain (Lorenz *et al*. 2009) and, despite numerous studies worldwide, costs of calf diarrhoea remain high, compared to other diseases on-farm (Younis *et al.* 2009).

Risk factors have been well documented in Europe and the UK as catalysts for calf diarrhoea in dairy herds (Bendali *et al.* 1999; Bazeley 2003). New Zealand dairy farming systems have been less well examined and the literature is limited. This literature review (Chapter 1) outlines the major risk factors for calf diarrhoea, based predominately on international research, while Chapter 2 focuses on the identification of risk factors for pre-weaning calf diarrhoea in the New Zealand context, with a view to offering some concrete advice to farmers for prevention and control.

1.1 Case definition of pre-weaning calf diarrhoea

Diarrhoea is defined as 'a rapid movement of fluids through the intestine resulting in poor absorption of water, nutritive elements and electrolytes' (Parkinson *et al*. 2010). Under normal circumstances, most fluid which enters the intestine is resorbed, but an intestinal abnormality can decrease fluid absorption due to damage of the intestinal wall villi by infectious organisms or the osmotic effect of nutritional overload. Diarrhoea may also occur with increased intestinal motility.

This dissertation examines risk factors for pre-weaning calf diarrhoea in New Zealand, meaning diarrhoea in any form (i.e. consistency 'custard like' to 'watery') and of any aetiology in calves from birth to weaning. 'Scouring' is a colloquial term used by New Zealand farmers in reference to diarrhoea, particularly in calves. 'Enteritis' is defined as inflammation of the intestinal tract and usually results in diarrhoea. The term 'gastrointestinal disease' pertains to any disease that affects the gastrointestinal tract and may also result in diarrhoea. For the purposes of this literature review, 'scours' and 'diarrhoea' will be used interchangeably. Any reference to 'gastrointestinal disease' or 'enteritis' assumes a strong association between these and the presence of diarrhoea.

'Neonatal' refers to infant calves of 1 to 7 days of age, but up to 28 days old. This study explores diarrhoea in pre-weaning calves and will include calves beyond 'neonatal' age.

1.2 Causative agents for pre-weaning calf diarrhoea

Aetiological diagnosis of the cause of calf diarrhoea may not be relevant for the treatment of the individual, but does aid prevention at the herd-level, especially if zoonotic pathogens are involved (Millemann 2009). As potential risk factors are the main predictors of interest in this study, the different aetiological agents for calf diarrhoea will not be examined. Although pre-weaning diarrhoea in calves is common in New Zealand (Vermunt 2002), clinical differentiation of infectious agents is difficult, with faecal cultures necessary to isolate the causative agent (McDougall and Cullum 1999; Vermunt 2002). Moreover, recent work by Constable (2010) suggests that faecal cultures may not truly reflect the population of small intestinal organisms.

Australasian research (McDougall and Cullum 1999; Vermunt 2002; Parkinson *et al*. 2010), as well as several overseas studies (Schroeder *et al.* 1985; Svensson *et al.* 2003; Izzo *et al*. 2011), have identified Rotavirus as the major causal pathogen for infectious diarrhoea in calves. Different pathogens and different species of pathogen vary in their virulence (Howe *et al*. 2008), with between-country variability in the pathogens responsible for calf diarrhoea.

In general, Coronavirus infection, although present in New Zealand, appears to be of little consequence in calf diarrhoea (Vermunt 2002). The same, minimally pathogenic relationship between Coronavirus and calves has also been noted in the Netherlands (Bartels *et al*. 2010). *Escherichia coli* (*E.coli)* infections are an uncommon cause of diarrhoea in New Zealand and are largely limited to calves less than five days of age (Vermunt 2002). A recent Dutch study showed that the prevalence of *E.coli* and Coronavirus infection was low (only affecting one or two calves) when compared with *Clostridium perfringens* bacteria (Bartels *et al.* 2010). Ninety-six percent of Swedish herds investigated for calf diarrhoea (Silverlas *et al.* 2009) were found to be infected with Cryptosporidia.

It should be noted that an association has been demonstrated between the presence of the intestinal pathogens and the presence of diarrhoea in calves, but these enteropathogens may not always necessarily cause diarrhoea (Bartels *et al*. 2010).

Mixed infections with Rotavirus and Cryptosporidia (Eschrig *et al.* 2004; Bartels *et al*. 2010), Rotavirus and *E.coli* (Younis *et al.* 2009) or Rotavirus and Salmonella are relatively common overseas (Clark and Gill 2001). Most outbreaks of calf diarrhoea in dairy and dairy beef operations in an Australian study involve multiple pathogens, with Rotavirus and Cryptosporidium most frequently identified (Izzo *et al*. 2011). Similarly, in New Zealand, the cause of diarrhoea is often complex and usually involves an interplay between enteropathogenic bacteria (Vermunt 2002). Multiple infections may also result in more severe disease (McDougall and Cullum 2003).

1.3 Incidences of morbidity and mortality due to pre-weaning calf diarrhoea

Incidence risk is defined as the number of new cases in a cohort during a defined time period divided by the total number 'at risk' at beginning of the time period**.** The incidence rate is the number of new cases in a cohort during a defined time period divided by the total 'animal time at risk' during the time period.

The incidence risk of morbidity ('morbidity risk') due to diarrhoea in calves less than 30 days of age is between 15.0% and 20.0% (Lorino *et al.* 2005). The incidence risk of mortality ('mortality risk') secondary to diarrhoea in pre-weaning calves varies between 1.5% and 8.0% (Lorino *et al.* 2005).

Sivula *et al*. (1995) reported a morbidity rate due to enteritis of 0.15 cases per calf days at risk, with a mortality risk secondary to diarrhoea of 17.9%. The incidence of calf enteritis in a 1982 study (Blom *et al.*) was 10.6%, while Curtis *et al.* (1988) noted a 9.9% crude morbidity risk from diarrhoea between birth and 14 days of life. In comparison, Waltner-Toews (1986) reported a substantially higher morbidity risk between birth and weaning of 20.5%. Two Swedish studies (Svensson *et al.* 2003 and 2006) reported a morbidity rate of 0.035 cases per calf month at risk and morbidity risk due to calf diarrhoea of 2.7%.

Gitau *et al.* (1994) showed a crude calf morbidity and mortality risk of 27% and 22% per year respectively, where diarrhoea was the most common cause. This was similar to a crude mortality risk documented by Perez *et al.* (1990) at 24.6% and a study in 2010 (Bartels *et al*.) that reported 19.1% of calves with diarrhoea and 23.8% of calves with 'custard like' faeces.

No estimates for calf morbidity and mortality risk are available for New Zealand, which reinforces the necessity and relevance of the current study.

Table compare morbidity and mortality for calf diarrhoea from studies cited, adjusted for the risk period in this study (birth to weaning at 70 days)

			Reported			Adjusted		
Author	Year	Country	Risk period (days)	Morbidity	Mortality	NZ weaning age	aMorbidity	aMortality
Blom	1982	Scandinavia	56	0.297	0.148	70	0.371	0.185
Waltner-Toews	1986	Canada	140	0.205	0.038	70	0.103	0.019
Curtis	1988	Ithaca, U.S.A.	90	0.052	0.035	70	0.040	0.027
Perez	1990	NL	120	0.246	0.049	70	0.144	0.029
Sivula	1996	Minnesota, U.S.A.	112	0.150	0.179	70	0.094	0.112
Svensson	2003	Sweden	90	0.098		70	0.076	
Lorino	2005	France	120	0.144	0.042	70	0.084	0.025
Svensson	2006	Sweden	105	0.027		70	0.018	
Bartels	2010	NL	22	0.191		70	0.608	

1.4 Diagnosis of calf diarrhoea

There is considerable variability in the reported incidence of diarrhoea in calves, depending on whether the diagnosis is made by a veterinarian or a farmer (Blom *et al.* 1982; Svensson *et al*. 2006; Silverlas *et al.* 2009). Clinical examination by veterinarians will likely increase the sensitivity of studies to detect diarrhoea and other diseases, including more chronic disease processes (Svensson *et al.* 2003). Frank and Kaneene (1993) reported that an increased number of routine veterinary farm visits were associated with a decreased incidence of calf diarrhoea. Although this may have been related to an overall improvement in disease control strategies, this trend was not consistent across all study herds. In larger herds, an increase in the incidence of calf diarrhoea was reported, potentially due to an improvement in recognition of diarrhoea by the farmer and an increase in disease diagnosis due to increased observation by the veterinarian.

1.5 Importance of calf diarrhoea- economic and zoonotic implications

As approximately one-third of the cost of rearing a calf from birth to first calving is incurred during the first 12 weeks of rearing (Schouten 2003), calfhood diseases have a major impact on the economic viability of cattle operations, due to direct costs of calf losses, treatments and the long-term effects on performance (Donovan *et al.* 1998; Millemann *et al*. 2009; Lorenz *et al.* 2011). The average cost associated with the treatment, prevention and mortality of gastrointestinal disease has been reported to be US$33.46 per calf per year (Bendali *et al*. 1999). Data from a 1975 study conducted in the United States suggested that enteric pathogens kill up to 25% of calves per year, resulting in US$250 million in losses (Frank and Kaneene 1993). It is likely that costs associated with calf diarrhoea have increased in the last 20 years. Diarrhoea in calves less than 30 days of age is difficult to prevent as it is multifactorial, both in relation to aetiological agent and risk factors associated with management.

In addition to its economic impact, calf diarrhoea is important because of its public health implications. Many of the organisms causing diarrhoea in calves, including Salmonella and Cryptosporidia species, in calves are zoonotic and people with impaired immune function are especially at risk of contracting disease (Vermunt 2002; Lund *et al.* 2011). Enterotoxigenic *E.coli* is a significant cause of diarrhoea among children and travellers in the developing world (Younis *et al.* 2009). As farmers attempt to control diarrhoea in their calves through the administration of antibiotic-based treatments, there are also developing issues around antibiotic residues in food producing animals (Frank and Kaneene 1993; Dey *et al*. 2003), which may have further public health implications.

2. Risk factors for calf diarrhoea

Worldwide, there have been numerous studies conducted on risk factors for development of calf diarrhoea in dairy and beef herds (Perez *et al.* 1989; Frank and Kaneene 1993; Gitau *et al.* 1994; Bendali *et al.* 1999; Bazeley 2003; Svensson *et al.* 2003 and 2006). However, although a number of different risk factors have been identified as significant, the external validity of these results (i.e. the validity of extrapolating results to other populations) is problematic, due to differences in data analysis and statistical approaches (Silverlas *et al.* 2009).

Identification and prioritisation of the relevant risk factors associated with calf diarrhoea, in conjunction with a clinical diagnosis of the aetiological agent, may allow accurate advice to be given to farmers in order to help control this insidious condition. It is intended that this will result in substantial improvements in the welfare of calves, whilst providing economic benefits to the farmer as well as public health benefits.

The principles of preventing calf diarrhoea are aimed at either minimising the infectious pressure and/or at boosting the calf's immunity and resistance to disease (Bazeley 2003). However, the multifactorial nature of calf diarrhoea makes separation of the individual effects of different management factors challenging (Schuuman *et al.* 1990; Bendali *et al.* 1999) and advice to farmers should take this into account (Crouch *et al.* 2001; Younis *et al.* 2009).

2.1 Boosting the calf's immunity to disease

Colostrum management and feeding is commonly regarded as the single most important factor in the prevention of calf diarrhoea (Donovan *et al*. 1998; Bendali *et al.* 1999; Berge *et al.* 2005; Trotz-Williams *et al*. 2008). However, Sivula *et al.* (1995) found no significant difference in the mortality and morbidity of animals that had received adequate colostrum intake relative to those that had received inadequate intake.

Due to the structure of the bovine placenta, the calf is born without protective immunoglobulins (Igs) and, therefore, depends on the successful passive transfer of maternal Igs via colostrum (Parkinson *et al.* 2010; Lorenz *et al.* 2011) until endogenous Igs are produced. The time from birth to first feed, volume, temperature and the concentration of Igs in the colostrum (good quality colostrum contains at least 50g/l IgG or more) all influence the efficacy of disease protection (Vasseur *et al*. 2009; Parkinson *et al*. 2010; Furman-Fratczak *et al.* 2011).

Calves that lack adequate passive immunity have increased mortality rates and are more susceptible to the major calfhood infectious diseases, such as respiratory disease and diarrhoea (Blom *et al*. 1982; Donovan *et al*. 1998; Svensson *et al.* 2003). Although it has not been a consistent finding in all studies of calf diarrhoea, there is typically a reason for the apparent lack of association between good quality colostrum feeding and reduced incidence of diarrhoea, such as insufficient data variability or all farmers enrolled in the study were practicing good colostrum management so calves had sufficient serum Ig levels (Frank and Kaneene 1993; Lorino *et al*. 2005; Perez *et al*. 1990). However, it should be noted that, even where colostral management is good, a number of other factors may interplay to cause diarrhoea in the young animal.

2.1.1 Failure of passive transfer

Collecting blood samples from calves 24 hours to 7 days old is a cost-effective and efficient method to evaluate the failure (or otherwise) of passive transfer of gamma immunoglobulin (IgG) via colostrum, through the measurement of serum protein or gamma glutamyl transferase (GGT) levels. Subsequently, appropriate management practices can be recommended to reduce the risk of development of calf diarrhoea and progress can be monitored (Vermunt 2002). Although testing for IgG in calf serum is costly, this is the gold standard test for failure of passive transfer. Therefore, testing calf GGT or serum protein levels is more commonly undertaken by veterinarians in New Zealand. In New Zealand, serum protein greater than 5.5g/dl indicates adequate passive transfer (Vermunt 2002). However, this result may be influenced by dehydration levels. GGT levels of greater than 700U/l are considered adequate and levels of less than 200U/l indicate failure of passive transfer (Vermunt 2002). Similarly, serum IgG of less than 10g/l are considered inadequate (Vermunt, 2002; Furman-Fratczak *et al.* 2011).

Both New Zealand and international studies, involving blood sampling of calves, indicate that 30 to 60% can be hypogammaglobulinaemic (Anderson 1983; Vermunt *et al.* 1995 and 2002; Wesselink *et al.* 1999), although between-farm variation may be high. Calves with adequate serum IgG concentrations have been proven to have better health status, achieved bodyweight and were first inseminated sooner (Furman-Fratczak *et al.* 2011).

Poor calf vigour (which may or may not be due to dystocia) may predispose to failure of passive transfer and diarrhoea (Lorino *et al.* 2005) particularly if the dam is a heifer (Furman-Fratczak *et al.* 2011). A New Zealand-based colostrum feeding trial, found that feeding of IgG-rich colostrum was associated with reduced mortality in calves with diarrhoea. However, no significant difference was detected in liveweight gain between treated and control calves (*p*-value=>0.2) (Anderson 1983; McDougall and Cullum 2003). In other studies, calves with low IgG levels had mortality rates as high as 40% and depressed growth rates by 70 to 90g per day (Schouten 2003). Supplying an adequate amount and concentration of colostrum within six to 12 hours of birth to dairy heifers may enhance their future productivity (DeNise *et al.* 1988).

2.1.2 Time from birth to first feed

Early feeding with adequate volumes of warmed colostrum in the first six to 12 hours of life is vital to calf-rearing success as the calf's digestive tract becomes impermeable to large IgG molecules by 24 hours of life (McGuire *et al*. 1976; Bush and Staley 1980; Matte *et al.* 1982; Jaster 2005; Lorenz *et al*. 2011).

2.1.3 Volume of colostrum fed

Current recommendations in New Zealand and Australia are to feed new-born calves 10 to 15% of their bodyweight, preferably within the first six hours of life (Vermunt 2002; Jaster 2005). Typically, in New Zealand, new-born calves receive two litres of colostrum within the first six hours after birth (Vermunt 2002; Parkinson *et al.* 2010). Recently in Poland, Furman-Fratczak *et al.* (2011) stated that calves should ingest at least two litres (about 5% of their bodyweight) at first colostrum feeding. In comparison, for large Holstein-Friesian calves in Ireland, it is recommended that calves are given three litres of good quality colostrum within two hours of birth (Lorenz *et al*. 2011), while in North America, it has been suggested that calves should receive four litres of colostrum in order to provide adequate passive immunity in two separate two litre feeds, one within two hours of birth and the other within 12 hours of birth (Vermunt *et al*. 1995; Lorenz *et al.* 2011).

Regardless of the country-specific recommendation, as calf abomasal capacity is limited to 1.5 to 2 litres, smaller feeds more frequently are a necessity in the early stages of life. Feeding of colostrum beyond the initial 24 hours may also have advantages, as IgG can bind to infectious agents in the gut, limiting disease prevalence and severity (McDougall and Cullum 2003; Lorenz *et al*. 2011).

2.1.4 Colostrum storage

It is recommended in New Zealand that colostrum is stored in a lidded drum and stirred regularly (Anderson 1983). This should minimise the chances of spoilage bacteria, such as *Pseudomonas,* proliferating in the colostrum.

2.1.5 Twice a day or once a day feeding

Twice-a-day feeding is the norm on many New Zealand dairy farms, particularly as overloading the abomasum through over-feeding is a real risk in animals fed once a day. Calves switched to once-a-day feeding after being fed twice daily on liquid feed are at particularly high risk (Lorenz *et al*. 2011). An Irish study by Gleeson *et al*. (2008) found that milk feeding of calves on a once daily basis tended to reduce the total labour input per calf and did not adversely affect calf performance. However, more group outbreaks of diarrhoea were observed in calves on once-a-day feeding in a cold milk feeding trial (Gleeson *et al.* 2007), with these outbreaks coinciding with periods of cold weather. Current European legislation dictates that calves must be fed twice daily (Lorenz *et al.* 2011) and be given access to fresh water. Regardless of feeding frequency, traditional feeding levels of 8% of bodyweight may predispose larger, heavier calves to malnutrition, immunosuppression and disease, as they require a higher bodyweight proportion for rapid growth (Lorenz *et al.* 2011).

2.1.6 Temperature of colostrum

Colostrum and milk warmed to 39 degrees Celsius and fed twice daily is the most 'natural' way to feed calves in New Zealand (Anderson 1983; Vermunt 2002). Although feeding cold milk or once-a-day feeding have been used to reduce labour input, these practices have been anecdotally reported to predispose calves to diarrhoea by slowing feeding and disrupting digestive function (Schouten 2003). Notwithstanding this, there is a lack of strong evidence in New Zealand demonstrating the protective effects of feeding warm milk.

2.1.7 The concentration of immunoglobulins in colostrum

Specific IgG present in colostrum may protect against the more common enteropathogens causing calf diarrhoea, such as Rotavirus, Coronavirus and *E.coli*. Although vaccination of the dam prior to calving may boost colostrum IgG concentrations (Crouch *et al.* 2001; Heckert *et al.* 2005; Lorenz *et al.* 2011), this does not negate the need for adequate colostrum feeding. On some farms, it may be difficult to distinguish the benefits of vaccination versus simple changes in colostrum feeding, since where vaccination is implemented, careful colostrum feeding is commonly subsequently employed.

2.1.8 Induced cows, sick cows and heifers

Individual cow-level variation in colostral IgG concentrations, including a positive association between increasing parity and colostral quality, has also been suggested. In New Zealand, cows that have been induced to calve up to 12 weeks early have colostrum IgG levels inversely proportional to the degree of prematurity of the calf (Anderson 1983). Moreover, a Swedish study (Svensson *et al.* 2003) indicated that calves receiving colostrum from first lactation cows had higher odds of developing diarrhoea. This has also been suggested by other work (Perez *et al.* 1990), since colostrum from first parity dams may contain fewer IgG than colostrum from older cows.

Animals should be milked as soon as possible after calving since IgG concentrations degrade with time (Jaster 2005). Colostrum should not be fed from cows that have 'leaked' (i.e. dripped milk due to an inadequate teat plug) pre-calving and the use of colostrum that looks watery is also not recommended (Anderson 1983).

2.1.9 Force feeding colostrum

There is some conflicting evidence as to whether force-feeding colostrum, rather than allowing animals to feed out of a bucket or from a nipple feeder as soon as possible after birth, reduces the incidence of calf scours (Lorenz *et al*. 2011). Regardless, it is not recommended to leave the calf to suckle normally on the dam since it is difficult to ascertain how much colostrum has been ingested (Svensson *et al*. 2003). Svensson *et al.* (2003) also found that calves that received colostrum through suckling the dam had a significantly higher risk of developing severe diarrhoea relative to calves that were given their first meal of colostrum by the farmer. The authors hypothesized that this was likely due to a delayed time to first ingestion of colostrum and inadequate volumes of colostrum ingested by calves that were left on their mothers.

In New Zealand, dairy calves are normally removed from the dam within 12 to 24 hours of birth (Woods 1999). In a recent Canadian study (Trotz-Williams *et al*. 2008) the odds of failure of passive transfer were significantly higher in calves on farms where more than 75% of cows were allowed to remain with their calves for more than three hours post-calving, relative to farms where dams and calves were separated within three hours of birth. Roth *et al.* (2009) found that calves reared on their mothers were more likely to be in poorer health (predominately caused by diarrhoea) than calves reared on automatic feeders, but this did not result in an increase in veterinary treatment in this group and the mother-reared group still showed increased weight gains when compared with their artificially reared counterparts in the milk-fed period. This is presumably due to calves contracting enteric pathogens from their dams. In French beef calves, new-born animals had a higher hazard for diarrhoea where dams were not clean (i.e. covered in faecal material or mud), reinforcing the potential influence of the dam as a source of infection for neonatal calves (Lorino *et al.* 2004)

2.1.10 Checking calves receive enough colostrum

Checking that calves receive sufficient colostrum early in life is vital for their health and wellbeing (Schouten 2003). New Zealand-based research proved that only 80% of calves learnt to suckle within the first six hours of life and if calves failed to learn to suckle by the sixth hour then they also failed to learn in the subsequent 24 hours (Weselink *et al.* 1999). Heifers with serum IgG concentrations of greater than 10g/L at 30 to 60 hours of life have been shown to reach bodyweights that allow first insemination sooner and have a better health status overall (Furman-Fratczak *et al.* 2011).

2.1.11 Training the calves to drink

Sucking behaviour has been proven to be adversely affected by lack of contact between infant and dam (Roth *et al.* 2009). As successful colostral and milk transfer to the calf relies on the ability of that calf to suck effectively, it is important to train neonatal calves to suck. Therefore, time and labour costs associated with training new-born animals to feed from artificial feeders are cost-effective.

2.1.12 Milk replacer vs. whole milk feeding

There has not been a consistently reported effect of feeding milk replacer on the incidence of calf diarrhoea relative to the feeding of unpasteurised whole milk (Perez *et al.* 1990). Where a higher incidence of calf diarrhoea is reported in calves fed milk replacer, this may have been attributed to incorrect preparation of the milk replacer or quality issues (Anderson, 1983; Waltner-Toews *et al.* 1986). Moreover, anecdotal evidence suggests that since whole milk may contain infectious agents such as Rotavirus and Cryptosporidia, switching to a good quality calf milk replacer may be a useful tool in correcting variations in whole milk quality (Schouten 2003). Conversely, Gorka *et al.* (2011) showed a negative effect of milk replacer on the maturation of the small intestine in Polish calves, which had subsequent negative effects on rumen development. This in turn had negative effects on the growth and metabolic status of the animal. The authors reported that body weight losses are often observed in the first weeks after feeding milk replacer to new-born calves (Gorka *et al*. 2011).

Despite this, in New Zealand it is currently recommended that milk replacer in the form of a powder of the correct particle size should be given to neonatal calves, ensuring the product is mixed correctly (Anderson 1983).

2.1.13 Other additives in colostrum or milk

It is reasonably common in New Zealand for water to be added to colostrum to increase volume. Although it is prohibited to feed milk containing antibiotics to 'bobby' calves (those calves intended for sale at 4 days of age), it is common practice to feed milk that contains antibiotics to neonatal 'replacement' calves. Milk containing antibiotic residues interferes with the fermentation process in the calf's gut, but does not select for microbial resistance (Vermunt 2002). Fermented colostrum is not suitable as a source of IgG for new-born calves and it has been suggested that 'waste' milk should be pasteurised before it is fed (Vermunt 2002; Lorenz *et al*. 2009). It is also common practice in New Zealand to add preservatives to colostrum and stored whole milk, including citric acid, formic acid and hydrogen (Vermunt 2002).

2.2 Environmental influences on the development of calf diarrhoea

2.2.1 Farm management factors

2.2.1.1 Time allocated to calf rearing

Inadequate time allocated to calf rearing and inadequate labour may predispose to calf diarrhoea (Gitau *et al.* 1994; Bazeley 2003; Waltner-Toews *et al.* 1988). Gleeson *et al*. (2008) found that the total average time consumed by calf care per farm decreased (*p*- value <0.01) with herd size and the average time consumed per calf was highest in small herds (<50 cows, 2.1 minutes per day).

2.2.1.2 Stockmanship

New Zealand pasture-based systems do not lend themselves to easy observation of the new-born calf because calves are housed in group housing systems from birth and are then out at pasture. Larger herd sizes may also make it difficult to assess whether an infant has a good suckling reflex and has received adequate colostrum, because individual cows and calves may not be as closely observed in large groups. In the Northern hemisphere, where animals are principally housed and calved indoors, cows may be more strongly affected by the quality of stockmanship around parturition relative to cows calved more extensively in New Zealand paddocks (Bazeley 2003; Lorenz *et al.* 2011). Very few weak or sick calves are provided with specific individual care in New Zealand, including heat lamps and isolation pens, relative to that seen overseas. Nevertheless, good stockmanship in New Zealand is still important to ensure that calves receive adequate colostrum and maintain health through to weaning.

In general, it is recommended in New Zealand that calves are reared by one, dedicated care-giver, with that person solely responsible for the calf-rearing on that property. This is beneficial in terms of minimising pathogen transfer to the young animals and should allow more accurate disease detection. A recent Canadian study (Trotz-Williams *et al.* 2008) showed that total serum protein levels were significantly greater in calves cared for by female relative to male caregivers. On New Zealand dairy farms, calf caregivers are often female.

2.2.1.3 Herd size

Larger herd size has been positively associated with an increased incidence of calf diarrhoea in Michigan, USA (Frank and Kaneene 1993). This may be due to overstocking or because there is a greater probability of a large outbreak problem in a larger population (Frank and Kaneene 1993). Similarly, a retrospective study of 127 dairy farms in the Manawatu region of New Zealand found that calf mortality and morbidity also increased with herd size (Schouten 2003).

2.2.1.4 Breed associations

In a Swedish study, breed had a significant association with the incidence of diarrhoea in calves up to 90 days of age (*p*-value=0.019) (Svensson *et al*. 2003). Swedish Red and White calves had a higher incidence of disease than other Swedish breeds tested. Currently, there is no data to suggest a breed effect on the development of calf diarrhoea in New Zealand.

2.2.1.5 Navel disinfection

'Navel ill' is the colloquial term for omphalophlebitis or infection and inflammation of the umbilicus. It is biologically plausible that 'navel ill' in calves may be associated with an increased risk of calf diarrhoea since the two disease processes share common risk factors, such as poor hygiene. However, no effect of navel disinfection on the development of calf diarrhoea was noted by Waltner-Toews (1986) nor Lorino *et al*. (2005).

2.2.1.6 Weaning ages and calf management

Peculiarities of the New Zealand seasonal calving system include selling bobby calves from 4 days of age and weaning of spring born replacement calves at various times depending on weather and other management factors.

Although replacement calves in New Zealand are frequently weaned at six to seven weeks of age (Woods 1999), it is recommended that they are weaned on weights rather than age (InCalf for New Zealand dairy farms, 2007), particularly as a reduction in weaning weights can affect the long-term productivity and survival of cattle (McDougall and Cullum 2003). Replacement calves should gain about 0.7kg/day liveweight over the entire milk feeding period (Vermunt 2002).

Bobby calves are managed differently from replacement calves in that they are kept in a separate pen for ease of pick up. They are not fed antibiotic milk and cannot be treated with antibiotics. Bobby calves accidentally fed antibiotic milk must be fed 'clean milk' for at least seven days before they can be sent for slaughter. If a cow is treated with antibiotics during gestation and the calf is born within the withholding period of the drug, then the calf cannot be sent as a bobby calf until after the withholding period is complete. Hence, bobbies are usually sent at four days old, but may remain on farm for longer in some instances. Bobby calf management and removal from the farm at four days of age may affect the pattern of calf diarrhoea and the pathogens responsible for calf diarrhoea in this group of animals.

Replacement stock are usually born in the first month of the calving season, since farmers typically undertake artificial insemination for one month and then allow the bulls to mate the remaining cows. Calves born from bull matings are seldom kept as replacement animals. Data on weaning age of replacement calves in New Zealand is limited. Starter ration intake in the best method to determine the time of weaning rather than age or liveweight. Calves can be weaned when they are consuming 1-1.5kg/day of starter ration (Vermunt 2002). Weaning on the presence of a ‘pear-shaped’ abdomen (indicating rumen development), instead of weaning on weight or age is recommended (Schouten 2003). Anecdotally speaking, weaning age varies from farm to farm between 6 and 12 weeks of age in New Zealand spring calving dairy herds.

Logically, the longer the period of risk the higher the chances of calves being diagnosed with diarrhoea. For the purposes of this prospective study the period of risk will be assumed to be approximately equal for all bobby and for all replacement calves. The two groups will be split to run analysis.

2.2.2 Calf feeding practices

It has been suggested that milk intake via an artificial teat does not fully satisfy a calf’s motivation to display natural sucking behaviour (Roth *et al.* 2009). Recent work suggests that there is little difference between nipple bucket and simple bucket-fed calves in the development of calf diarrhoea, providing feeding equipment is properly cleaned (Lorenz *et al.* 2009). Moreover, calves reared on milk feeders (compared to calves reared on their mothers) have been shown to ingest less milk and, therefore, have a higher concentrate intake, better rumen development and were more likely to be able to cover their energy demands with solid food only post-weaning (Roth *et al.* 2009).

However, where calves are fed from a large *ad lib* drum with teats, there is some controversy over the ideal number of calves per teat. Some authors specify that there should be some competition generated between the calves and six to eight calves per teat is ideal (Anderson 1983). However, where competition is too fierce, lighter animals will not be able to compete for feed and will lag further behind the mob (Anderson 1983).

2.2.2.1 Hygiene of calf feeders

Feeding equipment and utensils may be contaminated and unhygienic if they are not cleaned after use. There has been some work done suggesting that bucket-fed calves are more prone to calf diseases, especially those of a respiratory nature (Anderson 1983). This may be because calves are more likely to inhale milk when their heads are down in bucket fed situations, as opposed to when they are in a more 'natural' feeding position on teats.

2.2.2.2 Automated feeding systems

Nutritional scours are relatively common in the New Zealand system (Vermunt 2002) and have also been described overseas (Bazeley 2003). Ingestion of too high a volume of milk (Roth *et al.* 2009) or incorrect preparation of milk replacers (wrong concentration or temperature) may lead to nutritional scours (Anderson 1983). Feeding at irregular intervals or starvation followed by 'greedy' feeding (Bazeley 2003) may also contribute to a nutritional diarrhoea, although the aetiology is uncertain. No enteric organisms are cultured from true cases of nutritional diarrhoea, but nutritional scours may predispose animals to subsequent infectious scours (Bazeley 2003).

Although automated feeding systems may reduce labour requirements (Gleeson *et al*. 2008), observation and diagnosis of disease may be limited and, if the machine is set too low, either purposefully or accidentally, animals may starve. Moreover, as with all group feeding systems, smaller calves may be denied access to feed. It has been found that, compared with calves reared with unrestricted access to their mothers, calves reared on automatic feeders were more likely to display 'cross-sucking' behaviour (Roth *et al.* 2009). 'Cross-sucking' is defined as one calf sucking the ear, mouth, scrotum, prepuce, tail, udder area or navel of another calf. Calves given relatively limited access to their mothers, at 15 minutes twice daily, were less likely to display 'cross-sucking' behaviour than calves reared on automatic feeders. Marce *et al.* (2011) found that the risk of enteric and respiratory diseases increased with within-pen variability in calf age if automatic milk feeders were used, indicating younger calves were not receiving adequate milk due to between-calf competition with older calves.

In the author's experience, computerised and automated calf feeding systems are rare in New Zealand. This may mean that calf-rearers have a more accurate idea of the amount of feed ingested by an individual calf than where automated systems are used.

2.2.2.3 Supplementary feeding

In addition to their 10 to 12% body weight liquid-feed ration, supplementary feeding in young calves is recommended to stimulate rumen development (Vermunt 2002), with solid feed intake considered the main stimulant (Gorka *et al*. 2011). Although roughage feeding is also reported to be a protective factor against diarrhoea (Waltner-Toews 1986), no effect of additional concentrate feeding on the incidence of calf diarrhoea was found by Perez *et al*. (1990). Cocciodiostat is routinely added to commercial calf meal in New Zealand (occasionally in questionable quantities). Coccidiostats are ionophore antibiotics which prevent calves from developing Coccidiosis, a parasitic disease of the intestinal tract which may lead to diarrhoea, usually at grazing.

Although it has been suggested that even very young calves will ingest some hay if offered (Vermunt 2002), Lorenz *et al.* (2011) have stated that starter concentrate intake is negligible in the first three weeks of life.

A good starter ration contains 20% crude protein (Vermunt 2002). Liquid feed is relatively expensive compared with meal and roughage, so it is in the interest of the farmer to wean calves promptly. However, this may be to the detriment of liveweight gains. Underfed, early weaned animals may be predisposed to diarrhoea due to the immunosuppressive effects of malnutrition (Lorenz *et al.* 2011). Calves should only be weaned when they are consuming 1 to 1.5kg/day of meal (Vermunt 2002).

2.2.2.4 Fresh water access

Calves should be given free access to clean water under the Animal Welfare Act, 1999 (parts 1 and 2). Although, anecdotally, it appears few farmers in New Zealand allow milk-fed calves access to fresh clean water, *ad lib* access to clean water has been shown to augment dry feed intake (Vermunt 2002).

2.2.3 Calf housing

2.2.3.1 Group or individual housing

Group housing, where calves are penned in groups of two or more, is believed to better meet the behavioural and social needs of the animals than individual housing. Although group housing with automatic feeding has been shown to reduce the incidence of diarrhoea, this may simply reflect difficulties in detecting disease (Svensson *et al*. 2003; Svensson *et al.* 2006).

In group situations it is often difficult to detect the point source of diarrhoeic disease if many animals are affected, since the first signs of disease are often divergence of the animal from the main group (Svensson *et al*. 2006; Furman Fratczak *et al*. 2011). Group housing has been reported as a risk factor for calf morbidity, particularly where animals are housed in large group pens of up to 30 calves (Waltner-Toews *et al.* 1986; Perez *et al.* 1990; Svensson *et al.* 2003 and 2006; Marce *et al*. 2010).

It has long been recognised that individual housing for calves allows for control of infectious loads and reduces the incidence of clinical disease, compared with group housing (Davis *et* *al*. 1954; Perez *et al*. 1990; Marce *et al*. 2010). Frank and Kaneene (1993) found that individual maternity areas reduced the risk of calf diarrhoea. This is presumably due to a reduction in transfer of infectious pathogens between calves, as well as the extra attention afforded to animals housed individually (Svensson *et al.* 2003).

European legislation sets a maximal age limit (8 weeks) for raising calves in individual pens, acknowledging the necessity for calves to have social contact with other animals (Marce *et al*. 2009). Calf hutches are used to individually house animals, but allow adequate physical contact.

In New Zealand, dairy calves are normally reared by hand in group situations. Typically, calves spend the first week or two in a shed affording shelter, with the remainder of the rearing period typically at pasture (Woods 1999). The majority of enteropathogens in new-born calves are acquired from the dam, the calving break (at pasture) or the calf pens (Vermunt 2002). A Swiss study of dam-reared versus artificially-reared calves showed that, while artificially-reared calves more frequently displayed abnormal sucking behaviour, dam-reared calves had poorer health (predominately due to diarrhoea) (Roth *et al*. 2009). Interestingly, the overall number of animals that needed veterinary attention did not differ between the two groups (Roth *et al*. 2009). Weight gains during the milk-fed period were better for dam-reared calves than for artificially-fed calves, despite their ‘poorer’ health status. Permanent or restricted contact between the calf and its dam was found to have behavioural advantages by preventing abnormal ‘cross- sucking’ behaviour, although calves may be easier to handle and are conditioned to human contact if they are removed from their dams promptly. Dam-rearing of calves is seldom possible in the New Zealand context, indeed in dairying worldwide.

There is general agreement that calf housing in New Zealand should be north-facing and draught-free with dry, plentiful, untreated sawdust or similar bedding type (Anderson 1983; Vermunt *et al*. 2002). Partitions between calf pens should be solid and animals of similar age should be grouped together (Vermunt 2002), as mixed-aged housing and overcrowding predispose to diarrhoea and other calfhood diseases (Marce *et al*. 2011).

2.2.3.2 All-in-all-out housing policy

All-in-all-out policies are considered best-practice to minimise pathogen build-up in the environment, especially in non-seasonal systems. This may not always be practical in New Zealand calf-rearing operations, due to the seasonal calving pattern of New Zealand herds resulting in a continuous supply of newborn calves for around three months of the year. It is recommended that pens are thoroughly cleaned and disinfected between batches of calves (Vermunt 2002). A Swedish study found that using a continuous housing system or mixing a continuous and all-in-all-out housing system had a significantly higher prevalence ratio for Cryptosporidiosis than using an all-in-all-out system (Silverlas *et al.* 2009).

2.2.3.3 Calf area and stocking density

The minimum floor area of calf housing in New Zealand must be $1.5m^2$ per calf, with two to three squared metres ideal (Vermunt 2002). A Swedish study found that pen area was significantly associated with neonatal calf diarrhoea (Svensson *et al*. 2006), with calves in smaller pen areas (6.2 to $<12.6m^2$) at four to 10 fold higher odds of developing diarrhoea than calves in larger pens ($>12.6m^2$).

2.2.3.4 Hygiene in the calf sheds

Hygiene is of the utmost importance in any calf housing system and irregular changing of bedding materials may predispose calves to diarrhoea (Perez *et al*. 1990; Younis *et al*. 2009; Marce *et al*. 2011). Consistent cleaning of housing (after removal of calves) has been found to provide a protective effect against Coronavirus (Bartels et al., 2010).

Commensal and infectious organisms can proliferate rapidly in an environment where hygiene is poor (Clark and Gill 2001). Newborn calves had a higher risk of diarrhoea when stalls were not cleaned after the calving season in French beef herds (Lorino *et al*. 2005) and Schummann *et al.* (1990) showed that poor drainage could be associated with an increased risk of mortality in dairy calves in Alberta, Canada. In the Northern Hemisphere, regular removal of bedding from group maternity areas between calvings and calving cows in individual pens reduced the risk of calf diarrhoea (Lorenz *et al*. 2009). It has also been shown that removal of the calf from its dam within an hour post-birth reduces the risk of neonatal calf diarrhoea by reducing the number of pathogens that the neonate is exposed to on the mother's coat and udder (Lorenz *et al.* 2009).

Ammonia concentrations in calf housing have been associated with an increased hazard of calf diarrhoea (Lorino *et al*. 2005). There is also a positive association between incidence of respiratory disease and incidence of diarrhoea in calves, presumably because both diseases are linked to poor hygiene.

2.2.3.5 Environmental longevity of enteropathogens and disinfection

Coccidial oocysts are resilient in the environment and may survive for considerable lengths of time, with high humidity and moderate temperatures facilitating the survival of sporulated oocysts. Other micro-organisms may also survive for long periods if an appropriate disinfection regimen is not employed. Appropriate disinfection is the use of a disinfectant registered and proven to kill enteric organisms in the environment, applied for the recommended contact time and preferably after removal of all calf bedding materials which may harbour disease causing organisms. Rotavirus displays marked environmental resistance (Clifford *et al.* 2009) and may survive for up to 9 months (Clark and Gill 2001). Salmonella species can persist in calf rearing units for up to two years (Vermunt 2002). Cryptosporidia oocysts are highly resistant to commonly used disinfectants and readily persist in the environment (Vermunt, 2002). Recent work from Sweden linked poor disinfection of single calf pens to Cryptosporidial shedding in the main herd (Silverlas *et al*. 2009).

2.2.3.6 Location of calf housing

Most dairy farmers in New Zealand supply milk to a large corporate dairy company called Fonterra. Fonterra recommends that calf housing should be more than 20 metres away from the milking shed. Although there is no published data to support this practice, reducing contact between calves and adult animals should reduce the transfer of pathogens from older to younger stock, thereby reducing the incidence of diarrhoea in calves.

2.2.3.7 Bedding materials

It has been suggested that calves should be housed on packed sawdust or wood shavings from timber that has not been chemically treated (Vermunt 2002). Anderson (1983) suggested that sawdust is the best bedding for neonatal calves as it provides reasonable drainage and warmth.

2.2.3.8 Bedding cleaning and replacement

Removal of all bedding and cleaning of calf sheds between seasons is best practice. There has been some evidence to suggest that cleaning before each calving season and cleaning after each diarrhoeic episode may actually lead to diarrhoea (Lorino *et al*. 2005). One explanation for this is that farmers who have experienced diarrhoea in the past may be more likely to clean and disinfect calf housing. Silverlas *et al.* (2009) found that cleaning single-calf pens less frequently reduced the prevalence ratio of *Cryptosporidia* infection. This may have been because oocysts are covered by deep litter bedding and removal of bedding exposes the oocysts and, therefore, the calves to infection.

In an earlier study, regular removal of bedding from the maternity area between calvings was negatively associated with calf diarrhoea (Frank and Kaneene 1993). The removal of soiled bedding will likely remove a substantial portion of any faecal material harbouring infectious organisms.

The risk of disease associated with pathogens harboured in bedding has been explored by Hogan *et al.* (2007) with regards to bacteria commonly associated with mastitis. They found that bacterial counts differed between treated and untreated bedding immediately after disinfectant application, but that by day two to six after application, bacterial counts were similar.

2.2.4 Biosecurity and quarantine measures

2.2.4.1 Removal of sick animals from healthy ones

If sick animals are not removed and isolated from the main mob, they can act as a major source of infection. In particular, carrier animals, that do not show clinical signs, can continually shed pathogens into the environment (Bartels *et al.* 2010). Therefore, sick animals should be permanently removed from the healthy group, even if clinical signs have completely resolved (Vermunt 2002).

2.2.4.2 Quarantine policy

'Bought in' animals, especially from multiple sources, may introduce new disease pathogens to a property. To minimise this risk, it is best for farmers to rear their own replacements or to buy from recognised and trusted sources. Separation of bought in calves from those born on-farm is recommended to minimise the risk of pathogen transfer. It is anecdotally believed that New Zealand dairy farmers do not prioritize the quarantining of animals from outside sources from on-farm stock. This may be because the majority of New Zealand farmers breed their own calves and are not buying in stock.

2.2.4.3 Biosecurity measures

Minimising the spread of infectious pathogens from sick calves to healthy calves and from sick calves to humans requires, as a minimum, the employment of basic biosecurity measures. The routine cleaning of footwear and wearing gloves when handling stock are commonly recommended practices.

2.2.5 Previous diarrhoeic disease and individual calf factors

2.2.5.1 Historical incidence of diarrhoea

A previously high incidence of diarrhoea in calves on a farm was found to predispose to a high incidence of diarrhoea in subsequent seasons (Lorino *et al.* 2005). This may be related to transmission of pathogens from older to younger calves or to a lack of change in management practices meaning the between-season pathogen load in the environment remained constant (Bendali *et al.* 1999).

The odds of detecting Rotavirus in the faeces of Dutch calves increased 2.4 fold when one or more diarrhoeic calves were present at the time of sampling (Bartels *et al*. 2010). This makes biological sense due to the highly infectious nature of Rotaviral calf diarrhoea.

The risk of a second case of diarrhoea was higher in calves with a previous case of diarrhoea (Waltner-Toews 1986; Curtis *et al*. 1988; Perez *et al*. 1990) and Gitau *et al*. (1994) also found that an increase in calf mortality was associated with prior clinical illness.

2.2.5.2 Concurrent disease

Previous work has shown an association between diarrhoeic and respiratory disease in calves overseas (Curtis *et al*. 1988, Perez *et al.* 1990, Svensson *et al.* 2006). This may be due to common risk factors being responsible for the two infectious diseases or due to immunosuppressive effects of certain infectious agents predisposing animals to subsequent disease processes.

2.2.5.3 Age of calves

There is some suggestion that older calves are less susceptible to diarrhoea than younger calves (Curtis *et al.* 1988). Svensson *et al.* (2003) found that diarrhoea was diagnosed at a significantly lower age (median = 26 days) than respiratory disease (median = 52 days). Calves in the first two weeks of life were more likely to develop diarrhoea than their older counterparts in other overseas studies (Curtis *et al.* 1988; Perez *et al*. 1990; Frank and Kaneene 1993; Younis *et al*. 2009; Bartels *et al*. 2010). Similarly, Sivula *et al.* (1995) found that the risk of enteritis was highest in the first three weeks of life and a Dutch study found that the percentage of calves with ‘non-normal’ faeces was highest in the second week of life, compared to the first and the third week (Bartels *et al.* 2010). This could be because older calves are more commonly housed in groups (particularly in European farming systems), making it more difficult to detect disease (Svensson *et al.* 2006). This is also consistent with the aetiological agents responsible for diarrhoea in the neonatal calf, namely *E.coli* and viral infections, and with host immunocompetency (Curtis *et al*. 1988). In the Dutch work, calves of two weeks of age were more likely to test-positive to Rotavirus and Cryptosporidium compared with younger or older animals (Bartels *et al*. 2010).

In an African study, Gitau *et al*. (1994) found an unusual pattern of disease in which overall calf morbidity was higher in older calves.

2.2.5.4 Date of calf birth (early or late born)

Cold weather in the winter months has been documented as a potential predisposing risk factor for calf diarrhoea (Curtis *et al.* 1988; Frank and Kaneene 1993; Svensson *et al.* 2006). Month born may affect the incidence of calf diarrhoea, but this has not been well studied and requires more prolonged follow-up periods to provide conclusive data. However, month born may be compounded by other risk factors, such as stocking density and build-up of environmental pathogens (Lorino *et al*. 2005). Inadequate ventilation (leading to build up of ammonia) and inadequate drainage in calf housing may be a risk factor for calf diarrhoea and has been observed in overseas studies (Schuuman *et al.* 1990; Lorino *et al*. 2005). Dutch data suggests that the ‘outdoor season’ (April to October) is associated with a higher incidence of calf diarrhoea potentially related to an increased risk of hypothermia secondary to exposure (Perez *et al.* 1990).

Late-season born animals may face a higher infectious load and challenge as organisms ‘build up’ in the calf-rearing environment over time and clinically affected animals previously housed in the calf-rearing facilities shed high numbers of pathogenic organisms. Late-season born animals may also lack adequate colostral immunity due to insufficient availability of colostrum inherent with fewer cows calving at that end of the calving season which may compound the issue.

Calves born prematurely (at the start of the season) may also be predisposed to disease since premature calves often lack adequate passive transfer. This may be a function of lack of vitality (Furman-Fratczak *et al.* 2011), as calves born prematurely may be born weak and unable to suckle.

In New Zealand 'bobby calves' (regardless of timing of birth) are of low economic value compared with replacement heifers (2010 industry figures quoted $14 value for bobby calf) and are often not given the same level of care and attention as replacement stock and may, therefore, be predisposed to diarrhoea.

3. Treatment and control options for pre-weaning calf diarrhoea

3.1 Treatment options for calf diarrhoea

Calves with diarrhoea urgently require water and electrolytes to correct fluid imbalances (Schouten, 2004) and it has become increasingly evident over the past 20 years that treatment of calves with diarrhoea should not include withholding milk (Heath *et al*. 1989; Garthwaite *et al*. 1994). To reduce body weight loss in scouring calves, farmers are now advised to use rehydrating electrolyte therapy in the treatment of diarrhoea (Garthwaite *et al.* 1994, McGuirk *et al*. 1998). However, prolonged electrolyte therapy as the sole treatment of diarrhoea (i.e. without supplementary milk) can cause death through starvation.

An example treatment protocol would be to continue to feed milk at two litres twice daily, with supplementation of one to two litres of electrolyte, depending on the calf's level of dehydration (Vermunt 2002). The electrolyte should not be mixed with milk and the two feeds should be separated by at least 1.5 hours. Feeding oral electrolytes and milk together may interfere with casein coagulation in the abomasum (McGuirk *et al.* 1998). Many New

Zealand dairy farmers are still using the traditional treatment protocol of withholding milk from diarrhoeic calves for 24 hours and veterinarians seem to be failing to deliver this new message to their clients.

3.2 Antibiotic therapy for diarrhoeic calves

The common practice of treating scouring calves in New Zealand with antibiotic therapy should perhaps be questioned. Traditionally, it has been suggested that the most important and effective therapy for calf diarrhoea is replacement of fluid and electrolytes and that, by comparison, other therapeutic treatments have only minor impacts on calf losses (George 1987). Veterinarians usually choose not to prescribe antibiotics for the treatment of diarrhoea in neonatal calves without concrete evidence of bacterial aetiology. Schouten (2003) states that “antibiotics are of no value unless *Salmonella* is present”. Bartels *et al*. (2010) isolated Coronavirus in animals previously treated with antimicrobial therapy, suggesting that this opportunistic (and largely minimally pathogenic) enteric organism colonised the gut where antimicrobials were administered.

Anecdotally, current recommendations by most rural veterinary practitioners in New Zealand are that neither oral nor parenteral antibiotics should be used in the treatment of calf diarrhoea. The reasons for this are:

- the promotion of antimicrobial resistance,
- a substantiated claim that antimicrobials may alter intestinal flora and induce diarrhoea (which has been documented on more than one occasion with chloramphenicol, neomycin and penicillin), (Constable, 2004)
- a view that more ‘good’ bacteria than ‘bad’ are harmed in the gut by antimicrobials; and
- that antibiotics are not thought to be effective. (Constable 2004).

In 2003, Svensson *et al.* found that, in Sweden, 30% of cases of diarrhoea were treated with antibiotics (mainly oral), while only 19% of the cases received an oral electrolyte solution.

Constable has reviewed the use of antimicrobial therapy to treat diarrhoea in calves (2004, 2009). The basis for his suggestions is, regardless of the aetiology, calves with diarrhoea often have increased coliform numbers in the small intestine. He suggests antibiotic therapy is very useful where calves show signs of systemic illness (fever, inappetance or lethargy), particularly since 20 to 30% of systemically ill calves with diarrhoea have bacteraemia, largely due to *E. coli.* Since there are two sites of infection which need to be addressed in the diarrhoeic animal, the choice of antibiotic needs to ideally reach therapeutic levels in the blood and in the small intestine (excreted in an active form in bile).

Most importantly, antimicrobial therapy for calf diarrhoea must reach therapeutic concentrations at the site of infection for a long enough period and ideally only have a narrow Gram-negative spectrum of activity to minimise damage to other enteric species (Constable, 2004). In the author's opinion, it is challenging to ensure calves receive enough antibiotic (up to minimum inhibitory concentration; MIC) for a long enough course to be confident that farmers are not promoting antimicrobial resistance in calves with diarrhoea. Minimum inhibitory concentration is the concentration of antibiotic below which bacteria can proliferate. It is too tempting for the farmer in the busy spring period to truncate a course of antibiotics for a calf with scours that seems to have responded to the first dose of antibiotic. Furthermore, oral administration of potentiated sulphonamides is not recommended for treating calf diarrhoea because of the lack of efficacy studies (Constable 2004). Parenteral antibiotics in the form of ceftiofur, amoxicillin trihydrate or ampicillin trihydrate and potentiated sulphonamides can be recommended for use in calf diarrhoea. Potentiated sulphonamides have only been proven when treatment began before the onset of diarrhoea and ceftiofur use for calf diarrhoea is extra-label. It is worth reminding the farmer that calves with diarrhoea and no systemic illness should be monitored and not treated with antibiotics.

Parenteral administration of antibiotics is preferable to oral and the parenteral antibiotic of choice should also be bactericidal and predominantly Gram negative in spectrum (Constable 2009). *Per os* administration of chloramphenicol, penicillin and neomycin has been shown to increase the incidence of diarrhoea in healthy calves (Constable 2004). The use of an oral antibiotic with high oral bioavailability may reduce the risk of such deleterious effects (Constable 2004). There is still a dearth of peer-reviewed published data on the efficacy of antibiotics labelled for use in diarrhoeic animals, so it is proposed that off-label drug use may be valuable. No parenteral antimicrobial agents have a label claim for treating calf diarrhoea in the United States (Constable 2004) or in New Zealand.

One of the hallmarks of rotavirus pathogenicity in calves is the destruction of enterocytes lining the villi, resulting in blunted and fused villi (Clark and Gill 2001). Recovery depends on tissue repair and replacement of the cells lining the gut wall. Villous atrophy follows most infections that colonise the small intestinal cells so diarrhoea will continue after the causative agent has been eliminated (Bazeley 2003; Lorenz *et al*. 2011). This means that antibiotics, while they may act on some of the causative agents of the diarrhoea, do not provide the entire therapeutic solution.

In a survey in America in 1993 (Frank and Kaneene), only 21% of farmers relied on oral rehydration therapy in the treatment of scours and 80% still relied on antibiotics. It is hypothesised that this may also still be the case in New Zealand (Schouten 2004). Another American study has shown that minimising or eliminating the use of antibiotics in the feed requires measures to ensure adequate passive transfer, but in the face of inadequate passive transfer, animal welfare may be endangered by replacing medicated milk replacer with non-medicated milk replacer and therapeutic antibiotics with non-antibiotic alternatives (Berge *et al*. 2005).

3.3 Analgesic therapy for diarrhoeic calves

Interestingly, additional analgesic therapy (Flunixin meglumine and Meloxicam) for scouring calves is also beneficial. It is hypothesised that few farmers are routinely using analgesic medication in their diarrhoeic calves in New Zealand, despite Canadian and European evidence outlining the benefits. In the Canadian study, a single dose of Meloxicam resulted in early consumption of starter rations and improved starter ration intakes than their untreated counterparts (Constable, 2009). In the European study, a single dose of Meloxicam (in conjunction with electrolyte and antibiotic therapy) increased feed intake, hydration score, improved faecal consistency and decreased signs of visceral pain (Constable, 2009).

3.4 Oral electrolyte therapy

Maintaining the water, electrolyte and energy balance is the most critical factor affecting survival of the diarrhoeic calf (Schouten 2004). By comparison, other therapeutic treatments have only a minor impact on calf losses (George 1987). Dehydration of 12 to 14% in diarrhoeic calves is considered fatal.

3.5 Homeopathic therapy, probiotics and gut protectants

Although, homeopathic therapy for pre-weaning calf diarrhoea is increasing in popularity, particularly with the increasing number of organic dairy operations in Europe, none have been proven to be efficacious in peer-reviewed scientific literature (Constable, 2009). Similarly, intestinal 'protectants' and 'absorbents' have not been proven to be valuable (Constable, 2009).

3.6 Veterinary involvement in treatment decisions

Diarrhoea cases in Sweden (Svensson *et al*. 2003) were treated by farmers in 83% of cases, by veterinarians in 2% of cases and by farmers under veterinary recommendation in 15% of cases. It is hypothesised that clinical veterinarians and, in fact dairy industry in general, are failing to deliver coherent messages on the treatment options for calf diarrhoea in New Zealand.

3.7 Vaccination

Vaccination programs for young stock were positively associated with calf diarrhoea in America (Frank and Kaneene 1993). This may indicate that farmers place too much emphasis on vaccination to control disease in their young animals and may ignore other important management factors predisposing their animals to disease. Although Younis *et al.* (2009) demonstrated a clear protective effect of *E.coli* vaccination of the dam against the occurrence of *E.coli* diarrhoea in the calves, Bendali *et al*. (1999) found a negative association between dam vaccination with *E. coli* and calf diarrhoea despite a high prevalence of *E.coli* in the herds studied. However, the risk of calf diarrhoea has been shown to be higher in dams unvaccinated against rotavirus, bovine viral diarrhoea virus (BVDV) and coronavirus (Heckert *et al*. 2005). Therefore, the protection afforded by vaccination appears to be pathogen-specific. Since *E. coli* diarrhoea is relatively rare in New Zealand dairy calves compared to overseas and vaccination of New Zealand dairy cows for *E. coli* is rare, the questionnaire utilised in chapter 2 included whether farmers in the Waikato have vaccinated against rotavirus and salmonella only.

Two inactivated rotavirus vaccines are available in New Zealand. These vaccines work to reduce the incidence of scours, in calves fed colostrum from vaccinated animals, for the first two to three weeks of life (Anderson 1983; Vermunt, 2002). However, farmers must concurrently be reminded that calves still need to receive adequate and timely colostrum feeds and that the vaccine is not a 'silver bullet' solution for calf diarrhoea due to rotavirus infection in their herds.

Conclusions

Many risk factors have been identified as associated with morbidity and mortality due to calf diarrhoea. There is much published international work, but to the author's knowledge, there are no published studies based in New Zealand which prospectively investigate the risk factors for pre-weaning diarrhoea in dairy calves. The information compiled in this literature review was used to design a pre-calving questionnaire to identify management practices significantly associated with subsequent pre-weaning calf diarrhoea on 49 dairy farms in the Waikato region of New Zealand.

Study Design

The study hypothesis was to identify risk factors for pre-weaning calf diarrhoea (scours) on New Zealand dairy farms, using a questionnaire-based design.

In order to improve the external validity of the study, inclusion criteria for enrolled herds included a minimum herd size of 300 cows and spring-calving herds. Risk factors were examined at the herd-level, rather than at an individual calf-level. The outcomes of interest were:

- within-herd calf morbidity risk due to diarrhoea; and
- within-herd calf mortality secondary to diarrhoea.
- treatments for diarrhoea

Although the farmers that participated in the study were randomly selected, there was an element of voluntary participation. This meant that farms selected to participate may have been managed and run by motivated farmers who were more interested in productivity and in their animals' welfare than the average New Zealand dairy farmer.

Chapter Two: Risk factors for pre-weaning calf morbidity and mortality due to farmer-diagnosed diarrhoea on 45 New Zealand dairy farms

Materials and methods

A prospective observational study was undertaken to identify risk factors for pre-weaning calf diarrhoea on New Zealand dairy farming systems in the interval from birth to weaning. The defined time period for measurement of number of animals with diarrhoea was 1 July 2010 to 30 September 2010. Calves were split into 'bobby' and 'replacement' calves, with 'bobby' calves being sold at 4 days of age and 'replacements' being kept on farm as replacement stock. This allowed the time period at risk to be approximately equivalent for each of the groups examined.

A random number generator was used to select 70 farms from a client list of two branch practices of a large Waikato veterinary organisation (Anexa Animal Health). Sixty-five farms were sent a written invitation explaining the purpose of the study and telephoned to invite them to participate in May 2010. Sixteen clients declined to participate. Forty-nine farms with an average of 78 replacement calves per farm were enrolled in the study. This sample size was sufficient to determine a 10% prevalence among replacement calves with a 95% confidence interval of 9-11%, ignoring intra-farm correlation. The targeted 95% confidence interval was considered to be 2-3 fold larger as an unknown degree of intra-farm correlation was assumed to be present. The farms were all situated in the greater Hamilton area (37º47'S, 175º19'E). All farms enrolled were spring calving, between July 2010 and September 2010. Herd numbers ranged from 110 to 830, with a mean herd size of 380 cows. This is comparable to the wider population of Waikato farms (LIC New Zealand Dairy Statistics 2010/2011).

Study farmers completed a questionnaire describing their intended calf rearing practices for the upcoming calving period in May 2010 or June 2010. The questionnaire (Appendix 4) was developed following a literature review of reported risk factors for pre-weaning calf diarrhoea internationally, in addition to consultation with experienced New Zealand veterinarians. A total of 57 hypothesised risk factors were examined in the questionnaire. The questionnaire was pre-tested prior to trial start date for logic and length with four farmers, none of whom participated in the final study. Questionnaires were conducted by the first author (n=35) or a large animal technician (n=14) in a face to face interview with the farmer, 35 to 60 days before the planned start of calving in late June 2010 or early July 2010.

Each farmer was then provided with a standardised recording sheet ('Calf Treatment Log'- Appendix 3), to record all cases of diarrhoeic calves, which was designed to capture calf tag number, date of birth, date of treatment, treatment type, animal fate ('bobby' calf or 'replacement' calf) and whether the sick animal died. The standard study case definition was 'faeces with a consistency looser than normally observed in calves, watery faeces occasionally with undigested milk particles or blood'. Farmers were asked to record all diarrhoeic calves, irrespective of whether treatment occurred or not.

Farmers were also given a plastic bin and were asked to write the number of the animal treated on any treatment packaging used prior to disposal in the bin provided to allow for a comparison to be made between the number of treatments recorded and the number of treatments evident from the treatment packaging. Forty five farms provided information on numbers of sick calves but only thirty-eight (77.6%) study farms provided detailed product treatment information in both the bin and on the recording sheet. Although two farms provided farm-level treatment estimates, detailed individual animal level treatment information was not available. One farmer observed calves with diarrhoea, but did not record treatments given to these calves.

Enrolled farms were revisited approximately 10 weeks after the start of calving to collect the 'Calf Treatment Log'. The dimensions (length and width) of sheds used for calf rearing were measured using a metre wheel at this time. During the follow up period farmers were contacted on an informal basis during routine clinical visits to remind them of the data they were expected to collect for the study.

From the 'Calf Treatment Log' the cumulative incidence of calf diarrhoea between 1 July 2010 and 30 September 2010 (morbidity risk) and the cumulative incidence of calf death secondary to the observation of diarrhoea (mortality risk) were estimated for each farm. For 'replacement' calves, the morbidity/mortality risks were calculated between calving and weaning, while for 'bobby' calves, these risks were calculated between calving and removal from the farm.

A calf was defined as having had diarrhoea when diagnosed as such by the farmer/calf-rearer in the 'Calf Treatment Log'. Treatment packaging associated with calf diarrhoea was retrieved and used to verify the number of animals recorded on the treatment log.

The total number of calves on each farm was estimated from the total number of two year old (heifers) and mature (>two year old) cows due to calve on the farm. The number of replacement animals was captured in the questionnaire and the number of 'bobby' calves was defined as the difference between the estimated total number of calves born and the number of replacement calves.

Data analysis

The primary outcome variables were:

- Morbidity and mortality risk due to diarrhoea in pre-weaning bobby or replacement calves measured as the number of calves with diarrhoea, or dead due to diarrhoea respectively, until disposal or weaning divided by the number born alive;
- Remedies and administration routes used for treating pre-weaning calves with diarrhoea.

Statistical Analysis

Questionnaire and disease data were entered into a spread-sheet (Microsoft Excel 2010) and imported into the statistical software package Rv2.13 (R Development Core Team, 2009; The R Foundation for Statistical Computing, Vienna, Austria). Associations between the hypothesised risk factors and outcomes listed above were explored using bivariate and multivariable logistic regression. The unit of interest was a calf from birth to weaning. All risk factors were measured at farm level.

Some categorical variables were dichotomised where fewer than five farms were present in one or more categories and, where it was not logical to dichotomise the variable, some were excluded from further analysis. Distance of calf sheds from the milking shed (continuous variable) was dichotomised into 20 metres or less and over 20 metres. Calves were categorised into those defined as ‘replacement’ calves (predominantly female, but included eight bull calves kept to provide replacement bulls) or ‘bobby’ calves. ‘Bobby’ calves had a lower value than ‘replacement’ stock, were not treated with antibiotics (as they were entering the food chain) and were kept on-farm for a much shorter period than ‘replacement stock’ (thus having a much shorter time at risk).

The bivariate association between the hypothesised risk factors and the outcome variables were examined using chi-squared analysis and t-tests. Risk factors with a p-value of <0.01 were included in the multivariate logistic regression modelling (to reduce the risk of over-parameterisation of the multivariable models).

The degree of correlation between independent variables was assessed and, where variables were highly correlated (i.e. Pearson correlation coefficient of > 0.8), the most biologically plausible variable was selected.

The likelihood ratio test ($p<0.05$) was used to exclude variables using backwards elimination for the morbidity/mortality risk models. The likelihood ratio test was used instead of the Wald test to give a more conservative estimate of significance.

In order to account for over-dispersion and clustering at the herd level within the data set GEE (generalised estimating equation) multivariable models were fitted (Diggle et al. 2002). This procedure adjusted standard errors of risk factors for the correlation of morbidity and mortality of calves within herds.

The risk factors identified by the model were then incorporated into an on-farm risk assessment tool as a practical application of the risk factors identified in this study. The risk assessment tool is presented in Appendix 5 and was validated for ease of use with four veterinarians.

Results

Descriptive and bivariate statistics for the 45 herds in the study are presented in Appendices 1 and 2. A total of 17,178 calves were born on the 45 study farms. The reported mean replacement rate was 22.8% (range = 13.0% to 35.3%). Approximately 3,835 calves were kept as replacements, with the remaining 13,343 removed as ‘bobby’ calves at 4-12 days.

Pre-weaning calf morbidity and mortality risk due to diarrhoea

The length of the risk period for replacement and bobby calves were very different with bobby calves removed from the farm mostly at 4 days of age but occasionally up to 12 days of age. Replacement calves were kept on farm up until weaning time and so have a much longer risk period for developing diarrhoea. Most replacement calves (80%) were weaned between 8 and12 weeks of age. Unfortunately, individual calf weaning dates were not available since the weaning time was identified at the farm level in broad categories from the questionnaire (Appendix 4).

Calf diarrhoea was reported on 38 of the 45 (84%) farms that provided calf-level data. There was poor agreement (r=0.25, p=0.14) between the estimates of calf morbidity risk due to diarrhoea when based on clipboard reported cases compared to treatment packaging retained in the bins provided (Figure 1). Some farmers provided no disposed packages in the bins and the number of cases according to the bins was lower than those recorded on the clipboards (32/38, 84.2%, 95%CI=72.4%-95.9%). This was possibly because farmers used bottles of antibiotic for the calves and other bulk product that was not single use and so was not disposed of in the garbage bins. The numbers from the clipboards were used for analysis.

A total of 443 calves (2.6%; 95% CI =2.3%-2.8%) suffered from diarrhoea and 76 calves with diarrhoea (0.4%; 95% CI=0.3%-0.5%) died. This included 318 diseased and 35 dead replacement calves among 3,835 total replacement calves. A total of 125 out of 13,343 bobby calves at risk suffered from diarrhoea and 41 of these died. As shown in Figures 2 and 3, the ‘within-farm’ replacement calf morbidity risk ranged from 0 to 56.7%, while the ‘within-farm’ replacement calf mortality risk ranged from 0 to 6.7%.   The interquartile range of the risk of replacement calf diarrhoea (25-75% farms) was 12.8% (2.2% to 15.0%). The average replacement calf case fatality rate per farm was 11.9% (95% CI=4.2-19.2.8%, range 0-100%).

The total number of ‘replacement’ calves suffering from diarrhoea was significantly higher than bobby calves: 318/3,835 replacement calves (8.3%; 95% CI=7.4-9.2%) and 125/13,343 bobby calves (0.9%; 95% CI 0.8-1.1%) were recorded having diarrhoea before weaning or disposal, respectively. The farm-level morbidity risk in ‘replacement’ calves was 9.95% (95% CI= 6.3-13.6%) compared with 1.1% (95% CI=0.5-1.7%; p< 0.01) in ‘bobby’ calves. Similarly, the mortality risk was lower in ‘bobby’ compared to ’replacement’ calves (0.3%; 95% CI=0.1-0.5%) vs. (1.0%; 95% CI=0.5-1.6%); p=0.02). Although the case fatality rate was higher for ‘bobby’ than ‘replacement’ calves, the difference was not statistically significant either overall (41/125; 32.8% vs 35/118; 11%) or at the farm level (23.9%; 95% CI = 9.1-38.8%vs 11.9%; 95% CI = 4.5-19.2%; p-value=0.19).

After exclusion of collinear variables and including all variables which were significant at the bivariate level (p<0.01), there were fifteen and five variables included in the initial multivariable model for replacement calf morbidity rate and mortality risk, respectively. Eleven and five variables were included in the initial multivariable model for bobby calf morbidity rate and mortality risk, respectively

Model fit was assessed by examining the Pearson chi squared and deviance residuals. Pearson and deviance residuals lay within four standard deviations for the replacement morbidity risk and within 4 standard deviations for the replacement mortality risk. This was not ideal but was the best model fit given the set of covariates.

Risk factors associated with replacement and bobby calf morbidity risk due to diarrhoea at the bivariate level (p-value <0.01)

Fifteen variables were associated with replacement calf morbidity risk due to diarrhoea at the bivariate level. These are presented in Table 1. Eleven variables were associated with bobby calf morbidity risk due to diarrhoea at the bivariate level and these are shown in Table 2.

Risk factors regarding timing and volume of colostrum feeding were not significant at the bivariate level. The observed probability of replacement calf morbidity due to diarrhoea in herds where calves were fed less than or equal to 2 litres of volume was 9.2% compared with 7.4% in herds where calves were fed more than 2 litres of colostrum (p-value=0.05). The observed probabilities of replacement calf morbidity in calves which were fed colostrum at less than 24 hours old and calves which were fed colostrum at more than 24 hours old were 8.4% and 8.2% respectively (p-value=0.83). Farmers were also asked to record the time interval from calving to first milking (which may have given a better indication of the actual time to first feeding). The observed probability of replacement calf morbidity due to diarrhoea in herds where cows were milked at less than 12 hours after calving was 7.5%, compared with 8.6% in herds where cows were milked at more than 12 hours after calving (p-value=0.29).

Risk factors associated with replacement and bobby calf morbidity risk at the multivariable level (p-value<0.05)

Table 5 outlines the multivariable model for replacement calf morbidity risk. Replacement calf morbidity risk reduced by 28% for every 100 cow increase in herd size (p-value<0.01). Figure 4 confirms the relationship between herd size and the probability of diarrhoeic disease in replacement calves. Where calves were changed to once a day feeding at more than 3 weeks old, replacement calf morbidity risk decreased by 47%, compared with where calves were changed to once a day feeding at less than 3 weeks old (p-value=0.05). Where the number of teats per calf on the feeders changed as the calves get older, replacement calf morbidity risk decreased by 55% (p-value=0.02). Maternal vaccination pre-calving with rotavirus, salmonella or both rotavirus and salmonella vaccines was protective (odds ratio=0.38, p-value=0.02). Isolating sick calves in a different shed and feeding sick calves last increased the odds of replacement calf morbidity (odds ratios 3.25 and 2.77 respectively, p-value=0.01). Application of biosecurity measures in the calf sheds was protective, with gloves being the most highly protective strategy (p-value=0.02).

Table 6 outlines the multivariable model for bobby calf morbidity risk. Bobby calf morbidity risk decreased by 38% for every 100 cow increase in herd size (p-value<0.01) and the relationship is shown graphically in Figure 5. If more than one person was responsible for rearing calves, the odds of bobby calf morbidity increased. For two people the odds of calf morbidity was 3.62 times the odds of disease where one person was involved and for three people the odds of calf morbidity was 2.52 times the odds of disease where one person was involved (p-value=0.01). The use of navel spray and isolating sick calves in a different shed increased the odds of bobby calf morbidity (odds ratio 2.61 and 2.18 respectively, p-value=0.09 and 0.07).

Risk factors associated with replacement and bobby calf mortality risk secondary to diarrhoea at the bivariate level (p-value<0.01)

Five variables were associated with replacement calf mortality at the bivariate level (Table 3). Five variables were associated with bobby calf mortality at the bivariate level (Table 4).

In the final multivariable model, a decreased replacement calf mortality risk was associated with the time at which calves were put onto once a day feeding. Replacement calves put onto once a day feeding at more than 3 weeks of age had a reduced mortality risk of 79% (p-value<0.01). Where water was offered in the calf sheds the odds of replacement calf mortality reduced by 77% (p-value<0.01) compared to herds where no water was offered. A decreased replacement calf mortality risk was associated with changing the bedding in the calf sheds (odds ratio=0.15, p-value<0.01) compared with farms where the bedding in the calf sheds was not changed at all.

Bobby calf mortality risk decreased by 82% where water in the calf sheds was replaced daily or automatically refreshed compared to farms where no water was offered or the water was never changed (p-value<0.01). Mortality risk also reduced by 51% where the water was replaced every 2-3 days or more than every 2-3 days compared to where no water was offered or the water was never changed (p-value<0.01). Feeding bobby calves separately reduced bobby calf mortality risk by 88% (p-value<0.01) and feeding sick calves last reduced bobby calf mortality risk by 69% (p-value<0.01).

Treatment

Table 9 lists the treatments used for diarrhoeic calves by the 38 farmers who provided appropriate data.

Of the farmers which reported diarrhoeic calves, 81.6% used electrolyte therapy and 57.9% used antibiotics, either alone or in combination with electrolytes. Of the farmers which used antibiotics, 34.7% used oral, while 65.3% used injectable antibiotics.

Discussion

This is the first New Zealand study to quantify pre-weaning calf morbidity and mortality risk due to diarrhoea over multiple farms and to identify major risk factors for disease.

Incidence risk of morbidity and mortality

Our estimates of morbidity (10% for replacement calves) and mortality (1% for replacement calves) from this study were lower than those reported from other studies (see page 15 literature review), where the mean within-herd morbidity risk in pre-weaning calves was approximately 20% (range = 0, 70%) (Bendali *et al.* 1999 review). It was expected that the bobby calf morbidity risk may have been higher than the replacement risk due to poor husbandry and care of this group of animals. In fact, once the risk period was adjusted for bobby and replacement animals the rates were quite similar, although there was almost certainly under-reporting of the number of cases of diarrhoea in bobby calves. Interestingly, the case fatality risk was much higher in the bobby group than the replacement group which may simply indicate that farmers were more likely to euthanase sick bobby calves than treat them or, in fact, that the treatments given to bobby calves were sub-standard. Data from an American study in 1975 suggested that enteric pathogens killed up to 25% of the entire population of calves per year (Frank and Kaneene 1993). It is likely that the disease specific mortality has changed in more recent years, but this is similar to the case fatality risk seen in the bobby calf group in this study.

The lower mortality risk reported here may have been due to the significant differences in calf management systems found in New Zealand relative to other international dairy industries. Peculiarities of the New Zealand system are described in the introduction to this paper.

As indicated in the results section, there were risk factors which were expected to be of significance at both the bivariate and multivariable levels that were not significant at the nominated p-value, such as those associated with colostrum management. This may have been due to the different New Zealand management systems making risk factors of no consequence or due to confounding.

Potential issues with data

Completeness of data recording was an issue in this study. Moreover, there may have been under-reporting of the number of calves observed with diarrhoea possibly due to a lack of time during the busy spring period, unfavourable weather conditions or apathy. Over-reporting is unlikely to have been a problem in this study.

This was a prospective study, as participants were asked to complete a questionnaire prior to the calving season. Thus, the temporal relationship between the risk factors and disease occurrence are in the correct sequence. However, the management practices that the farmers said they were going to implement were only verified on an informal basis throughout the season via phone calls and farm visits for other clinical work. This could have introduced an element of 'response bias' to the study.

Additionally, some of the answers may have been biased by general best practice recommendations, rather than being reflective of actual management practices on farm. However, we believe that such response biases were minimised by employing a face-to-face interview. Some small interview bias might have been present since the face to face interviews were conducted by two different people.

The total number of calves in the population was estimated, by herd, based on the number of cows and heifers to calve provided in the questionnaire. This approach to estimating the total calf population could have introduced a source of bias as no direct measure of the number of calves born was undertaken. Moreover, there was no consideration given to animals which may have produced twins and/or lost a calf through late-stage abortion, stillbirth or dystocia. Therefore, the calf morbidity and mortality risk due to diarrhoea may be higher than what has been measured in this study. Data may further be biased by the fact that farmers who volunteered to participate could be higher achieving farmers, with better record keeping and higher milk production. Alternatively, they may have been particularly interested in calf diarrhoea due to a history of the disease on their farms.

In other studies, there is considerable variability in the reported incidence of diarrhoea in calves, depending on whether the diagnosis is made by a veterinarian or a farmer (Blom *et al.*1982; Svensson *et al*.2006; Silverlas *et al.* 2009). Clinical examination by veterinarians will likely increase the sensitivity of studies to detect diarrhoea and other diseases, including more chronic disease processes (Svensson *et al.*2003). Frank and Kaneene (1993) reported that an increased number of routine veterinary farm visits were associated with a decreased incidence of calf diarrhoea. Although this may have been related to an overall improvement in disease control strategies, this trend was not consistent across all study herds. In larger herds, an increase in the incidence of calf diarrhoea was reported, potentially due to an improvement in recognition of diarrhoea by the farmer and an increase in disease diagnosis due to increased observation by the veterinarian.

The aetiology of diarrhoea was not established in the current study. Hence, pathogen or aetiology-specific risk factors were not identified. Mortality risks calculated in this study did not distinguish between euthanasia and natural death, although it is assumed that animals which were euthanized would have died of natural causes in due course. This may not always have been the case, since some farmers are so fastidious about keeping enteropathogens out of their calf sheds that they euthanize all animals which develop any signs of disease immediately. This was possibly the reason for the relatively high case fatality risk in bobby calves in this study.

Time at risk played an important role in the diagnosis of calf diarrhoea. Eighty-two percent of the replacement calves were born within one month of the planned start of calving. Analysis of the bobby and replacement calves as two separate groups improved the accuracy of the mortality and morbidity risk calculations.

The multifactorial nature of calf diarrhoea makes assessment of the impact of individual risk factors difficult to quantify (Schuuman *et al.* 1990). Often multiple factors are associated with disease and advice to farmers should take this into account.

Risk factors of significance and interest

Several risk factors were identified as significantly associated with replacement calf morbidity. For every increase in 100 cows on the farm, the odds of disease decreased by 28% ($p<0.01$). This may be a function of larger farms having a dedicated calf rearer, purpose built facilities or more refined calf rearing management practices. Increasing herd size (by 100 cows) also reduced the odds of bobby calf morbidity by 38% (p-value<0.01).

Figures 4 and 5 reinforce the bivariate relationships between herd size and replacement and bobby calf morbidity respectively. There was a clear inverse relationship between the probability of diarrhoeic disease and increasing herd size.

Colostrum related risk factors were identified as being of significance in overseas work (McGuire *et al.* 1976; Bush and Staley 1980; Matte *et al.* 1982, Jaster 2005), but were interestingly not significant in this study.

On farms where calves were fed once daily feeding at an older age (more than 3 weeks of age) the odds of replacement calf morbidity and mortality due to diarrhoea decreased by 47% (*p*-value=0.05) and 79% ($p<0.01$) respectively. This may be because younger calves are less able to cope with large volumes of colostrum or milk in one feed since calf abomasal capacity is limited to 1.5 to 2 litres. Smaller feeds more frequently are a necessity in the early stages of life. Twice-a-day feeding is often the norm on many New Zealand dairy farms for very young calves, particularly as overloading the abomasum through over-feeding is a real risk for diarrhoea in animals fed once a day. Calves switched to once-a-day feeding after being fed twice daily on liquid feed are at particularly high risk of becoming ill (Lorenz *et al*. 2011).

Changing the ratio of the number of teats on the feeders, in relation to the number of calves in the mob, as the calves got older was associated with a decrease in replacement calf morbidity (p-value=0.05). In most cases (20/22, 91%) where the number of teats changed they were increased. This meant that calves were able to get enough liquid feed easily as the competition for teats was reduced and even the smaller calves were able to compete.

In our study 14 farms (31.1%) vaccinated their cows and heifers with either rotavirus vaccines, salmonella vaccine or both. Maternal vaccination pre-calving with rotavirus, salmonella or both rotavirus and salmonella vaccines, decreased the odds of replacement calf morbidity due to diarrhoea by 62% (p=0.02). In contrast, dam vaccination programs to protect young stock were associated with an increased incidence of calf diarrhoea in America (Frank and Kaneene 1993). This may indicate that farmers place too much emphasis on vaccination to control disease in their young animals and ignored other important management factors predisposing their animals to disease such as hygiene. It may also suggest that farmers who have had problems with calf diarrhoea were more likely to vaccinate their herds. Protection afforded by vaccination is pathogen-specific and vaccines work to reduce the incidence of diarrhoea, in calves fed colostrum from vaccinated animals, for the first two to three weeks of life (Anderson 1983; Vermunt, 2002). The protective effect of vaccination in this study tends to suggest that New Zealand dairy farmers who are vaccinating their herds are also aware of the importance and timely and adequate colostrum feeding. No faecal samples were collected from diseased calves and no pathogen information was collected for this study.

Further exploration of the data revealed that there were no significant associations between the time from calving to first feed and the time from calving to first milking and whether or not the herd was vaccinated. It is important that calves from vaccinated dams receive colostrum in a timely fashion post birth to ensure adequate transfer of antibodies from dam to calf via the colostrum (Vermunt 2002). It was surprising that dam vaccination did not have some correlation with timely colostrum transfer.

The replacement calf morbidity due to diarrhoea was higher on farms where sick calves were separated from healthy calves in a different shed (p=0.01). Increasing bobby calf morbidity due to diarrhoea was also positively associated with isolation of sick calves from healthy calves in a different shed, but when the GEE model was considered and the effect was averaged across the entire population it was no longer significant (although there was still a trend) (p=0.07). The standard errors are noticeably inflated for the GEE model and are therefore more conservative estimates.

Often farmers isolated sick animals in a separate pen, but allowed them to remain in the same airspace as their healthy counterparts, with or without a solid partition between healthy and sick calves. It is recommended that sick animals be removed completely from the group and housed in separate housing (Vermunt 2002). Carrier animals that do not show clinical signs can continually shed pathogens into the environment (Bartels *et al*. 2010). Some farmers moved calves with diarrhoea to a different shed, so housing in a separate shed cannot be a direct risk factor for sick calves, but rather a proxy for some other risk factor, such as historically high incidences of calf diarrhoea which may lead to 'best practice' isolation of sick animals and feeding sick animals after healthy ones. Despite the fact that isolation of sick calves was associated with an increase in the incidence of diarrhoeic disease in both bobby and replacement calves, it is possible that isolation and special treatment of sick calves may have meant they recovered more promptly from their diarrhoeic episode, but this was not measured. Since isolation is biologically unlikely to cause disease, reverse causality may be a problem here.

An increased replacement calf morbidity risk was associated with feeding sick calves last (p=0.01). While this result is counterintuitive, it may be assumed that sick pens are only created where there were large numbers of diarrhoeic calves, whereas if there were only one or two diarrhoeic calves then they remained with the main group. This could explain why on farms where there were more sick replacement calves, calves were more likely to be fed last. More logically, on farms where sick calves were fed last, diarrhoeic bobby calves were 69% less likely to die (p-value<0.01). Feeding sick calves last would likely minimise the transfer of pathogens to healthy calves and therefore reduce the risk of death associated with diarrhoea. Where calves were fed last it is also more likely that more time was spent with them which may have increased their chances of survival (Waltner-Toews*et al.* 1986; Bazeley 2003).

Rearing bobby calves separately either in separate pens or completely separate housing reduced the odds of bobby calf mortality by 88% (p=0.01). Since mortality in this study included euthanasia this may also be because bobby calves that were mixing with replacement claves were more likely to be destroyed due to diarrhoeic disease to prevent cross-infection between the two groups. Where bobby calves were reared in separate pens or sheds they are only mixing with calves of a similar age. Conversely, if bobbies were mixed with replacement calves they may be more prone to picking up pathogens from older calves or may not be given the opportunity to feed on group feeding drums due to competition from older animals which may predispose them to diarrhoea and subsequent death.

Biosecurity measures applied in the calf sheds were protective for replacement calf morbidity. On farms where calf rearers wore gloves, the odds of replacement calf morbidity was reduced by 81% (p-value=0.02), compared with where farms employed no biosecurity measures at all. Washing boots or footbaths at the calf sheds reduced the odds of replacement calf morbidity by 31% (p-value=0.02) compared with farms where no biosecurity measures were used. Basic biosecurity and hygiene measures should logically reduce the risk of disease and infectious pressure by reducing transmission of pathogens.

Water offered in the calf sheds reduced the odds of replacement calf mortality by 77% (*p*-value<0.01). Calves should be given free access to clean water under the Animal Welfare Act, 1999 (parts 1 and 2). *Ad lib* access to clean water has been shown to augment dry feed intake (Vermunt 2002), which may, in turn, encourage early rumenal development promoting maturity and immunocompetency. Thirteen of the 45 herds in this trial offered no water at all in the calf sheds and some relied only on paddock troughs to provide clean water to their stock. Additionally, on one farm, while water was offered in the shed, it was never refreshed. Since young calves are confined to calf sheds, paddock troughs are not available to these calves until they are older. Animals usually reject stale water and prefer access to clean fresh water to drink. In herds where water in the calf sheds was offered and replaced regularly, the odds of bobby calf mortality due to diarrhoea was reduced (p-value<0.01).

Hygiene is of the utmost importance in any calf housing system and irregular changing of bedding materials may predispose calves to diarrhoea (Perez *et al*. 1990; Younis *et al*. 2009; Marce *et al*. 2011). Consistent cleaning of housing (after removal of calves) has been found to provide a protective effect against Coronavirus (Bartels et al., 2010). Bedding replacement reduced replacement calf mortality by 85% (p-value<0.01) in this study.

Five farms (11.1%) did not replace bedding in the calf sheds at all. Fifteen farms replaced bedding between seasons; 4 farms replaced bedding during the season and 22 farms replaced bedding in the calf sheds both between and during the season. Replacement of calf bedding materials may not be prioritised in some herds' management strategies and high calf bedding costs may discourage regular replacement.

Commensal and infectious organisms can proliferate rapidly in an environment where hygiene is poor (Clark and Gill 2001). New-born calves had a higher risk of diarrhoea when stalls were not cleaned after the calving season in French beef herds (Lorino *et al*. 2005). In the Northern Hemisphere, regular removal of bedding from group maternity areas between calvings and calving cows in individual pens reduced the risk of calf diarrhoea (Lorenz *et al*. 2009).

Where more than one person was responsible for rearing calves the odds of bobby calf morbidity increased. If more than one person was entering the calf sheds on a daily basis to feed and care for the animals, the risk of introducing pathogens to the calf sheds on fomites such as boots increased. The standard and consistency of care for young calves may have suffered where more than one person was involved.

The use of navel spray in bobby calves increased the odds of diarrhoeic disease 2.61 fold.. Bobby calves may be more likely to be housed in sub-standard hygiene conditions than replacement calves. The use of navel spray may be an attempt to manage an unhygienic environment. Since hygiene risk factors for navel ill and diarrhoea are similar, it may make sense that farmers who used navel spray in an attempt to combat poor hygiene in their bobby calf pens were also more likely to suffer diarrhoeic disease in this group of animals. However, when averaged across the entire population (GEE model output), navel spray was no longer a significant risk factor for bobby calf morbidity (p-value=0.09).

Treatment Decisions

There were a wide range of products used to treat calves. As bacterial aetiologies of calf diarrhoea are relatively uncommon (Schroeder *et al.* 1985; Howe *et al.* 2008; Izzo *et al.* 2011), and as rehydration and correction of metabolic acidosis are the most important therapeutic goals (Lorenz *et al.* 2011), electrolyte therapy should be central to calf diarrhoea treatment. While over 80% of farmers did use electrolytes, many also used antibiotics in combination with electrolytes or alone. However, in a US study, calves receiving only non-antibiotic therapy were more likely to be in a lower health category compared with antibiotic treated calves (Berge *et al.* 2005).

Recent work by Constable (2004, 2009) suggests the therapeutic benefits of using antibiotic therapy in calves which were systemically ill (pyrexic and lethargic) due to diarrhoea. This is because 20 to 30% of systemically ill calves with diarrhoea have bacteraemia, largely due to *E. coli,* regardless of the cause of diarrhoea.

Conclusions

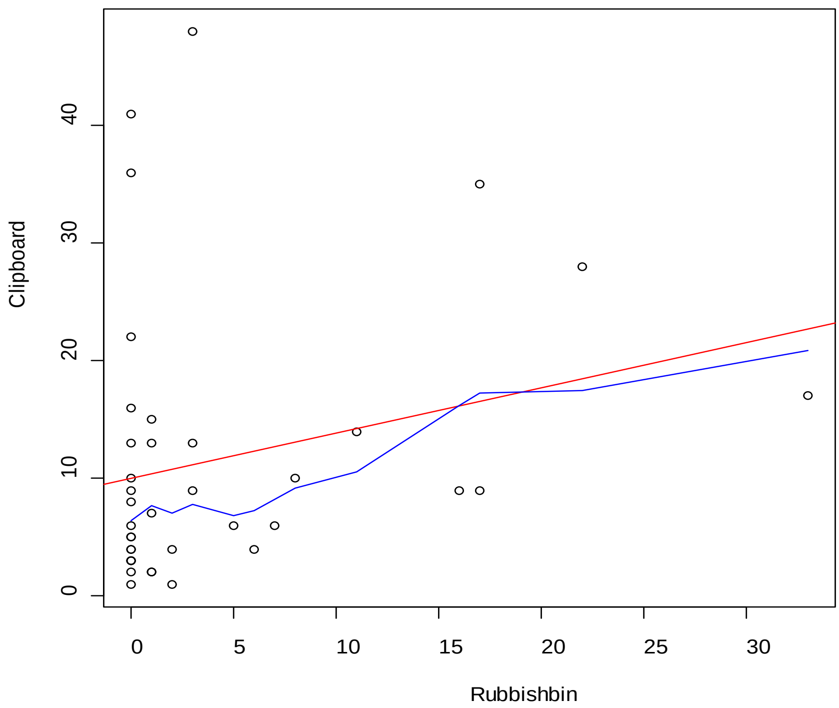
This is the first study of its kind in New Zealand to explore herd-level risk factors for pre-weaning calf diarrhoea. The estimate of calf morbidity risk due to diarrhoea was lower than many international studies. While this may partly due to under-reporting, it may also reflect the different management systems used in New Zealand relative to other systems internationally, such as early access to pasture, group housing systems with similar aged animals and removal of 'bobby' calves at 4-12 days of age from the rearing system.

Our study demonstrated that certain calf-rearing practices were significantly associated with an increased or decreased morbidity and mortality risk due to diarrhoea. While the study was not designed to indicate causality, some of the identified risk factors are biologically plausible and should be further investigated, such as fresh water access, timing of once a day feeding, isolation and hygiene and biosecurity measures.

The development of a risk assessment tool for use by clinicians, based on risk factors identified in this study, with their farmers pre-calving should provide a valuable insight into potentially risky management strategies and allow them to be modified accordingly.

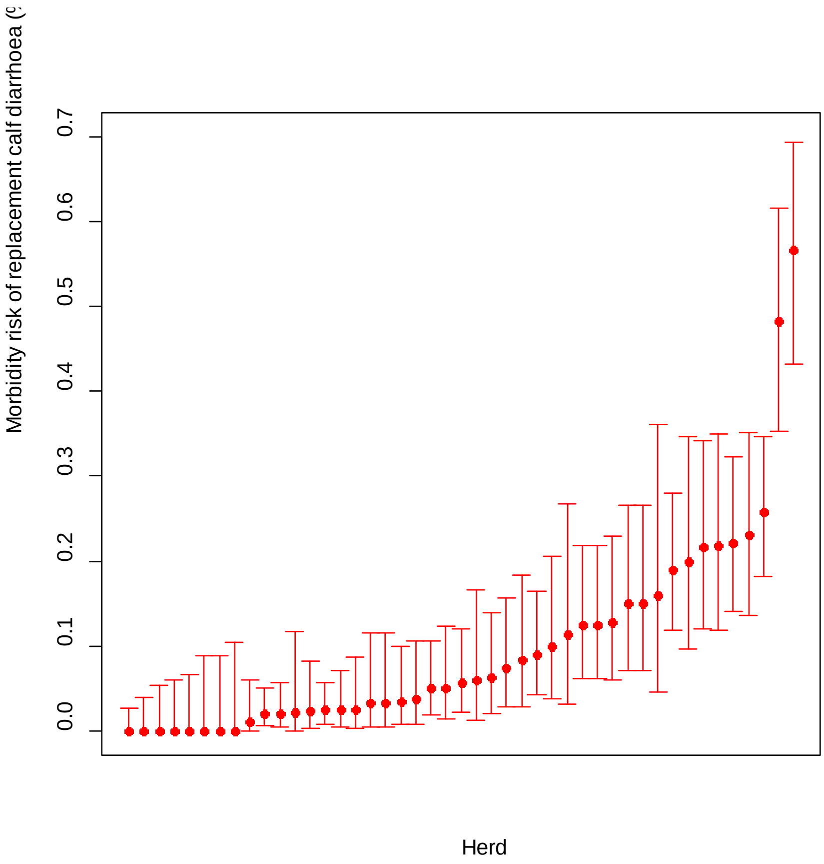
Figures

Figure 1: Comparison of estimating neonatal calf morbidity rates due to diarrhoea based on dairy farmer records ('clipboard') versus an audit of packaging for treatments typically used for calf diarrhoea ('rubbish bin')- blue line represents smoothed 'lowess' line and red line is line of best fit (r=0.25, p=0.14)



Each dot on the figure above represents a farm. There were more diseases calves recorded on the clipboards than rubbish in the rubbish bin. The clipboard recorded treatments were used as the outcome variable of interest

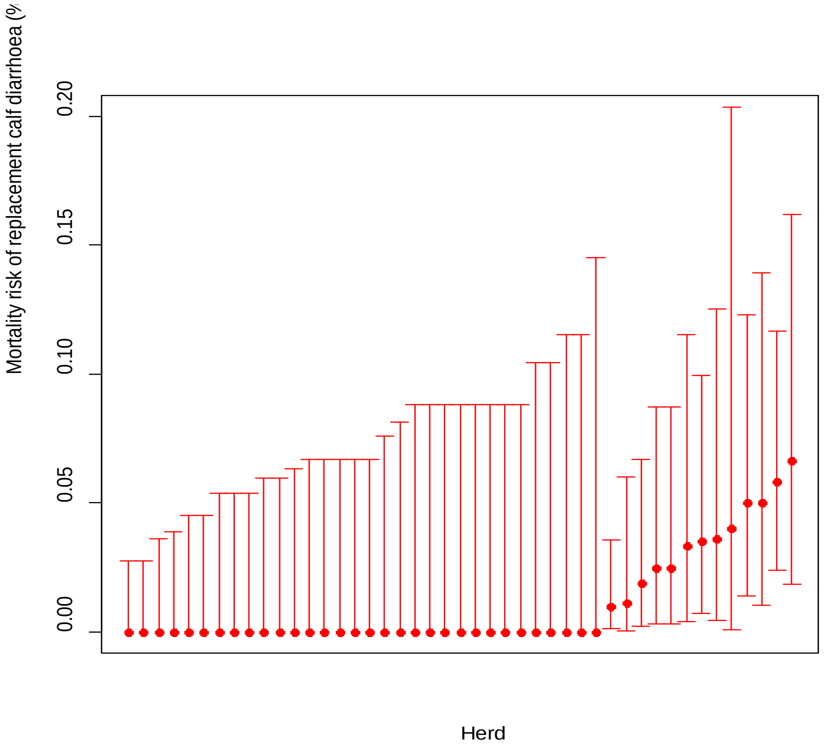
Figure 2: Herd averages of farmer reported pre-weaning incidence of replacement calf diarrhoea on 45 Waikato dairy farms, July –Sept. 2010 (in ascending order of incidence)



Median=5.7%

Interquartile range= 12.8% (2.2%-15%; 25%-75% of farms)

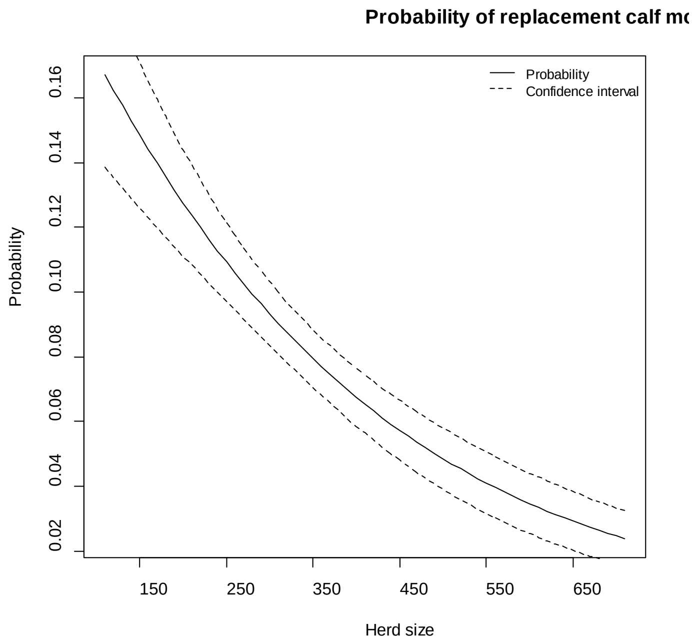
Figure 3: Herd averages of farmer reported pre-weaning mortality due to replacement calf diarrhoea on 45 Waikato dairy farms, July –Sept. 2010 (in ascending order of incidence)



Median=0%

Interquartile range 1.1% (0%-1.1%, 25% to 75% of farms)

Figure 4: Probability of replacement calf morbidity as a function of herd size

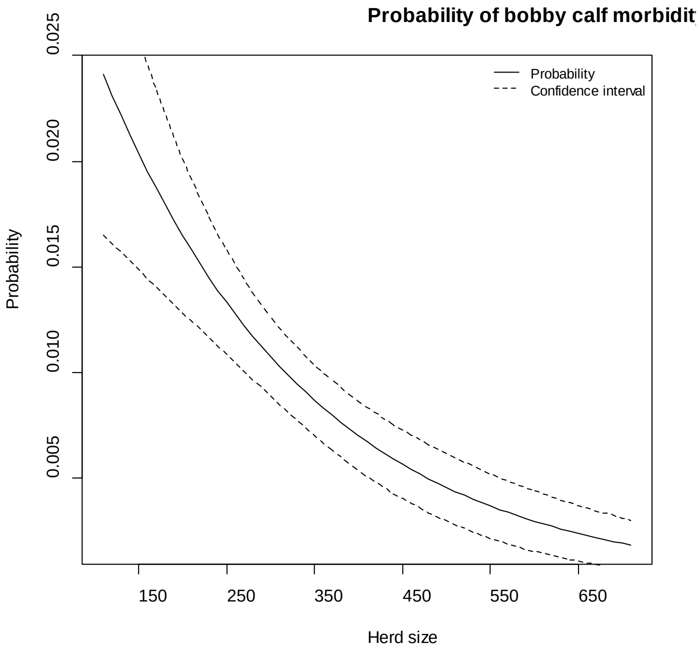


Beta coefficient= -0.0035, **odds ratio** (per 100 cows)=0.70

Interpretation: For every 100 cow increase in herd size the odds of replacement calf morbidity due to diarrhoea reduces by 30%.

Pearson's correlation coefficient =-0.28 (95% CI=-0.53,0.01), *p*-value=0.06

Figure 5: Probability of bobby calf morbidity as a function of herd size



Beta coefficient= -0.0043, **odds ratio** (per 100 cows)=0.65

Interpretation: For every 100 cow increase in herd size the odds of bobby calf morbidity due to diarrhoea reduces by 35%.

Pearson's correlation coefficient =-0.18 (95% CI=-0.45,0.12), *p*-value=0.23

Tables

Bivariate Models

Table 1: Crude associations (p<0.01) with replacement calf morbidity at the bivariate level

(n=3835)

Variable	Levels	Morbidity
Preservative added to colostrum	Yes	14.6%
	No	7.1%
Calves fed colostrum from sick/induced cows and heifers	Yes	10.9%
	No	5.6%
Time at which calves onto once a day feeding	Less than 3 weeks old	10.3%
	More than 3 weeks old	6.2%
Number of teats changes as calves get older	Yes	6.2%
	No	10.2%
Number of calf sheds	1	11.2%
	2	7.3%
	3 or more	8.3%
Calves moved between sheds	Yes	7.4%
	No	9.9%
Disinfectants used in calf sheds	Yes	9.5%
	No	6.1%
Disinfectant type used	None	6.1%
	Virkon	12.0%
	Other	7.0%
Timing of disinfectant use in calf sheds	None used	6.1%
	Betw seasons or during seasons	8.8%
	Both betw and during seasons	12.4%
Calf feeders cleaned between uses	Yes	9.4%
	No	6.8%
Navel spray used	Yes	10.2%
	No	4.5%
Maternal vaccination pre-calving (recat)	None	9.2%
	Rotavirus or Salmonella or both	6.5%
Sick calves isolated in a different shed	Yes	16.9%
	No	6.9%
Sick calves fed last	Yes	9.3%
	No	4.7%
Biosecurity measures applied in calf sheds	None	9.6%
	Gloves	3.5%
	Footbaths or washing boots	7.0%
	Gloves and footbaths or washing boots	11.5%

Risk factors fewer than 5 herds in one or more categories were excluded

Table 2: Crude associations (p<0.01) with bobby calf morbidity at the bivariate level (n=13334)

Variable	Levels	Morbidity
Preservative added to colostrum	Yes	1.5%
	No	0.8%
Colostrum warmed for first feed	Yes	0.7%
	No	1.3%
Calves fed colostrum from sick/induced cows and heifers	Yes	1.2%
	No	0.7%
Coccidiostat added to supplement feed	Yes	1.1%
	No	0.5%
Number of people rearing calves	One	0.5%
	Two	1.1%
	More than two	0.9%
Bobby calves reared separately	Yes	0.5%
	No	1.2%
Disinfectants used in calf sheds	Yes	1.1%
	No	0.6%
Disinfectant type used	None	0.6%
	Virkon	1.7%
	Other	0.5%
Navel spray used	Yes	1.2%
	No	0.5%
Sick calves isolated in a different shed	Yes	2.4%
	No	0.7%
Biosecurity measures applied in calf sheds	None	0.8%
	Gloves	0.4%
	Footbaths or washing boots	1.1%
	Gloves and footbaths or washing boots	2.0%

Risk factors fewer than 5 herds in one or more categories were excluded

Table 3: Crude associations (p<0.01) with replacement calf mortality at the bivariate level

Variable	Levels	Mortality
Time at which calves onto once a day feeding	Less than 3 weeks old	13.8%
	>3 weeks old	0.4%
Bedding replaced in the calf sheds	Yes	0.7%
	No	3.2%
Water offered in shed	Yes	0.5%
	No	1.7%
Frequency of changing water	Never changed/none offered	1.6%
	Daily/automatically refreshed	0.6%
	Every 2-3 days or >every 2-3 days	0.5%
Age calves go onto calfeteria	<3 weeks	1.4%
	>3 weeks	0.5%

Risk factors fewer than 5 herds in one or more categories were excluded

Table 4: Crude associations (p<0.01) with bobby calf mortality at the bivariate level

Variable	Levels	Mortality
Frequency of changing water	Never changed/none offered	0.5%
	Daily/automatically refreshed	0.1%
	Every 2-3 days or >every 2-3 days	0.4%
Number of calf sheds	1	0.9%
	2	0.2%
	3 or more	0.2%
Bobby calves reared separately	Yes	0.1%
	No	0.5%
Disinfectant type used	None	0.2%
	Virkon	0.6%
	Other	0.2%
Sick calves fed last	Yes	0.2%
	No	0.6%

Risk factors with fewer than 5 herds in one or more categories were excluded

Multivariable Models

Table 5: **Multivariable model results of significant risk factors for the incidence risk of <u>replacement calf</u> diarrhoea (45 New Zealand dairy farms)**

	GEE model				95% CI of OR	
Variable	**Estimate**	**SE**	***p***	**OR**	**lower**	**upper**
Model intercept	-1.19	0.46	0.01			
Number of cows to calve	-0.003	0.001	<0.01	0.72**	0.61	0.90
Time at which calves began OAD feeding						
less than 3 weeks old more than 3 weeks old	Ref. -0.64	0.32	0.05	0.53	0.28	0.99
Number of dummy-teats changed as calves got older No						
Yes	-0.79	0.33	0.02	0.45	0.24	0.86
Maternal vaccination pre-calving No						
Yes	-0.95	0.41	0.02	0.38	0.17	0.85
Sick calves isolated in a different shed No						
Yes	1.18	0.42	0.01	3.25	1.43	7.39
Sick calves fed last No						
Yes	1.02	0.41	0.01	2.77	1.23	6.23
Basic hygiene measures applied in calf sheds No biosecurity			0.02			
Gloves	-1.67	0.55		0.19	0.065	0.55
footbaths or wash boots	-0.37	0.48		0.69	0.27	1.77
gloves and footbaths or wash boots	-0.48	0.41		0.62	0.28	1.38

* OAD is once a day feeding;
** OR per 100 cows

Table 6: Multivariable model result of significant risk factors for the incidence risk of bobby calf diarrhoea from 45 farms in New Zealand

	GEE model				95% CI of OR	
Variable	**Coefficient**	**SE**	***P***	**OR**	**lower**	**upper**
Model intercept	-4.91	0.64	<0.01			
Number of cows to calve	-0.005	0.002	<0.01	0.62*	0.46	0.83
Number of people rearing calves One person	Ref.		0.01			
Two people	1.29	0.42		3.62	1.58	8.25
More than two people	0.92	0.61		2.52	0.77	8.25
Navel spray used No	Ref.					
Yes	0.96	0.56	0.09	2.61	0.87	7.85
Sick calves isolated in a separate shed No	Ref.					
Yes	0.78	0.43	0.07	2.18	0.93	5.05

* OR per 100 cows

Table 7: Multivariable model result of significant risk factors for the incidence risk of replacement calf mortality from 45 farms in New Zealand

	GEE model				95% CI of OR	
Variable	**Coefficient**	**SE**	**P**	**OR**	**lower**	**upper**
Model intercept	-1.89	0.54	<0.01			
Time at which calves onto OAD feeding- less than 3 weeks old	Ref.					
more than 3 weeks old	-1.56	0.58	<0.01	0.21	0.07	0.65
Water offered in shed No	Ref.					
Yes	-1.47	0.53	<0.01	0.23	0.08	0.65
Bedding replaced in the calf sheds No	Ref.					
Yes	-1.88	0.43	<0.01	0.15	0.07	0.35

*OAD is once a day feeding

Table 8: Multivariable model result of significant risk factors for the incidence risk of bobby calf mortality from 45 farms in New Zealand

	GEE model				95% CI of OR	
Variable	**Coefficient**	**SE**	***P***	**OR**	**lower**	**upper**
Model intercept	-3.97	0.25	<0.01			
Frequency of changing water in shed None offered or never changed	Ref.		<0.01			
Daily or automatically refreshed	-1.71	0.50		0.18	0.07	0.48
Every 2+ days	-0.72	0.41		0.49	0.22	1.08
Bobby calves reared separately No	Ref.					
Yes	-2.12	0.76	<0.01	0.12	0.03	0.53
Sick calves fed last No	Ref.					
Yes	-1.18	0.38	<0.01	0.31	0.15	0.64

Normal model estimates the particular effect of the particular variable in a specific herd
The GEE model SE are much inflated (more conservative) and indicates the effect of the predictor variable on the proportion of morbidity and mortality in calves across all herds in the study population

Table 9: Descriptive stats to show the different treatments used by farmers to treat calf diarrhoea on 38 farms in the Waikato region of New Zealand

Treatment	Number of farms	Percentage of Total
None	2	5.3
Injectable antibiotic	1	2.6
Oral antibiotic	2	5.3
Electrolytes	12	31.6
Electrolytes **Oral antibiotics**	3	7.9
Electrolytes **Injectable antibiotics**	7	18.4
Electrolytes **Oral antibiotics** **Injectable antibiotics**	5	13.2
Electrolytes **Homeopathy** **Injectable** **Oral antibiotics**	1	2.6
Injectable antibiotic **Probiotic**	1	2.6
Inert binder **Injectable antibiotic**	1	2.6
Electrolytes **Inert binder** **Injectable antibiotic**	1	2.6
Electrolytes **Inert binder**	1	2.6
Electrolytes **Naturopathy**	1	2.6

Group	Products
Electrolytes	Dexolyte Diarrest Electrolife Enerlect Glutellac Glycolect Revive
Naturopathy	Codliver oil Dolomite Hay tea Seaweed Silver Veratrum album
Inert binder	Bobby binder Rumenite Trubond
Injectable antibiotic	Betamox Bivatop Engemycin Intracillin
Oral antibiotics	Pink scour tablets Scourban
Probiotics	Biostart

References

Bibliography

1. **Abad F, Pinto RM, Bosch A.** Survival of Enteric Viruses On Environmental Fomites. *Applied and Environmental Microbiology* 60(10), 3704-3710, 1994
2. **Anderson D.** Proceedings of a course in Dairy Cattle Medicine. *Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA Annual Conference* 6-20, 1983
3. **Bartels CJM, Holzhauer M, Jorritsma R, Swart WAJM, & Lam TJGM.** Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. *Preventive Veterinary Medicine*, 93(2-3), 162-169, 2010
4. **Bazeley K.** Investigation of diarrhoea in the neonatal calf. *In Practice* 25(3), 152-159, 2003
5. **Bendali F, Sanaa M, Bichet H, & Schelcher F.** Risk factors associated with diarrhoea in newborn calves. *Veterinary Research* 30(5), 509-522, 1999
6. **Berge ACB, Lindeque P, Moore DA, Sischo WM.** A clinical trial evaluating prophylactic and therapeutic antibiotic use on health and performance of pre-weaned calves. *Journal of Dairy Science* 88, 2166-2177, 2005
7. **Blom JY.** The relationship between serum immunoglobulin values and incidence of respiratory disease and enteritis in calves. *Nordisk veterinaermedicin* 34(7-9), 276-284, 1982
8. **Clark G, Gill J.** Rotavirus in calves. *Vetscript* 14(11), 12-13, 2001
9. **Clifford C, Watson J.** Old Enemies, Still with Us after All These Years. *Ilar Journal* 49(3), 291-302, 2008
10. **Crouch C, Oliver S, Francis MJ.** Serological, colostral and milk responses of cows vaccinated with a single dose of a combined vaccine against rotavirus, coronavirus and Escherichia coli F5 (K99). *Veterinary Record* 149(4), 105-108, 2001
11. **Curtis CR, Erb, HN, & White ME.** Descriptive epidemiology of calfhood morbidity and mortality in New-York Holstein herds. *Preventive Veterinary Medicine* 5(4), 293-307, 1988
12. **Davis LR, Autrey KM, Herlich K.** Outdoor individual portable pens compared with conventional housing for raised dairy calves. *Journal of Dairy Science* 37, 562-570, 1954
13. **Diggle PJ, Heagerty P, Liang KY, Zeger SL.** Analysis of Longitudinal Data 2nd edition, New York: Oxford University Press, 2002
14. **DeNise S, Robinson JD, Stott GH, Armstrong DV.** Effects of passive immunity on subsequent production in dairy heifers. *Journal of Dairy Science* 72(2), 552-554, 1988
15. **Dey B, Thaler A, Gwozdz F.** Analysis of microbiological screen test data for antimicrobial residues in food animals. *Journal of Environmental Science and Health* B38(3), 391-404, 2003
16. **Dohoo IR, Martin W, Stryhn H**. *Veterinary Epidemiologic Research*, Prince Edward Island, Canada, 2003
17. **Donovan GA, Dohoo IR, Montgomery DM, Bennett FL**. Calf and disease factors affecting growth in female Holstein calves in Florida, USA. *Preventive Veterinary Medicine* 33, 1-10, 1998
18. **Donovan GA, Dohoo IR, Montgomery DM, Bennett FL.** Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Preventive Veterinary Medicine* 34(1), 31-46, 1998
19. **Eschrig M, Heckert, HP, Goossens, L.** Field trial to compare the effectiveness of two different dam vaccines (Rotavec (TM) Corona and Lactovac (R) C) against neonatal diarrhoea in cattle. *Praktische Tierarzt* 85(8), 580+-, 2004

20. **Faraway JJ**. *Extending the Linear model with R*. USA: Chapman and Hall/CRC, Taylor and Francis Group, 2006
21. **Farkas R, Szeidemann Z, Majoros G.** Studies on Coccidiosis of Calves in Hungarian Dairy Farms. *Parasitology Research* 101(1), S113-S120, 2007
22. **Frank NA, Kaneene JB.** Management risk factors associated with calf diarrhea in Michigan dairy herds. *Journal of Dairy Science* 76(5), 1313-1323, 1993
23. **Furman-Fratczak K, Rzasa A, Stefaniak T.** The influence of colostral immunoglobulin concentration in heifer calves serum on their health and growth. *Journal of Dairy Science* 94, 5536-5543, 2011
24. **Garthwaite BD, Drackley JK, McCoy GC, Jaster EH.** Whole milk and oral rehydration solution for calves with diarrhea of spontaneous origin. *Journal of Dairy Science* 77(3), 835-843, 1994
25. **George G.** Calf diarrhoea: fluid therapy. *Surveillance New Zealand*, 14(2), 5-6, 1987
26. **Gitau G, McDermott JJ, Waltnertoews D, Lissemore KD, Osumo JM, Muriuki D.** Factors influencing calf morbidity and mortality in smallholder dairy farms in Kiambu district of Kenya. *Preventive Veterinary Medicine* 21(2), 167-177, 1994
27. **Gleeson D, O'Brien B, Fallon RJ.** Feeding of cold whole milk once daily to calves in a group and its effect on calf performance, health and labour input. *International Journal of Applied Research in Veterinary Medicine* 5(3), 97-104, 2007
28. **Gleeson D, O'Brien B, O'Donovan K.** The labour input associated with calf care on Irish dairy farms. *Livestock Science* 116(1-3), 82-89, 2008
29. **Gorka P, Kowalski Z, Pietrzak P, Kotunia, A, Jagusiak W, Zabielski R.** Is rumen development in newborn calves affected by different liquid feeds and small intestine development? *Journal of Dairy Science* 94, 3002-3013, 2011
30. **Heath SE, Naylor JM, Guedo BL, Petrie L, Rousseaux, CG, Radostits OM.** The effects of feeding milk to diarrheic calves supplemented with oral electrolytes. *Canadian Journal of Veterinary Research*, 53(4), 477-485, 1989
31. **Heckert H, Bardella I, Brunner B, Brunner R.** Effects of maternal immunisation of cows- a field study. *Praktische Tierarzt* 86(7), 500-+, 2005
32. **Hogan JS, Wolf SL, Petersson-Wolfe CS.** Bacterial counts in organic materials used as free-stall bedding following treatment with a commercial conditioner. *Journal of Dairy Science* 90(2), 1058-1062, 2007
33. **Howe L, Sugiarto H, Squires RA.** Use of polymerase chain reaction for differentiation of Group A bovine rotavirus G6, G8 and G10 genotypes in the North Island of New Zealand. *New Zealand Veterinary Journal* 56(5), 218-221, 2008
34. **Izzo M, Kirkland PD, Mohler VL, Perkins NR, Gunn AA, House JK**. Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. *Australian Veterinary Journal* 89(5), 167-173, 2011
35. **Jaster EH.** Evaluation of Quality, Quantity and Timing of Colostrum Feeding of Immunoglobulin G1 Absorption in Jersey Calves. *Journal of Dairy Science* 88(1), 296-302, 2005
36. **Lorenz I.** An update on calf diarrhoea part 2: Prevention. *Irish Veterinary Journal* 62(2), 130-133, 2009
37. **Lorenz I, Mee JF, Earley B, More SJ.** Calf health from birth to weaning. I. General aspects of disease prevention. *Irish Veterinary Journal* 64, Article 10, 2011
38. **Lorenz I, Fagan J, More SJ.** Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. *Irish Veterinary Journal* 64(9), 2011.
39. **Lorino T, Daudin JJ, Robin S, Sanaa M.** Factors associated with time to neonatal diarrhoea in French beef calves. *Preventive Veterinary Medicine* 68(2-4), 91-102. 2005
40. **Lund B, O'Brien SJ.** The Occurrence and Prevention of Foodborne Disease in Vulnerable People. *Foodborne Pathogens and Disease* 8(9), 961-973, 2011
41. **Lundborg GK, Svensson EC, Oltenacu PA.** Herd-level risk factors for infectious diseases in Swedish dairy calves aged 0-90 days. *Preventive Veterinary Medicine* 68(2-4), 123-143, 2005

42. **Marce C, Guatteo R, Bareille N, Fourichon C.** Dairy calf housing systems across Europe and risk factors for calf infectious diseases. *Animal* 4(9), 1588-1596, 2010
43. **Martin SW, Meek AH.** A path model of factors influencing morbidity and mortality in Ontario feedlot calves. *Canadian Journal of Veterinary Research*, 50(1), 15-22, 1986
44. **Matte J, Girard CL, Seoane JR, Brisson GJ.** Absorption of colostral immunoglobulin G in the newborn calf. *Journal of Dairy Science* 65, 1765-1770, 1982
45. **McDougall S, Cullum A.** Effect of Feeding Colostrum of High Immunoglobulin Content on Calf Growth Rates and Disease. *Paper presented at the Proc 16th Annual Seminar, Society of Dairy Cattle Veterinarians NZVA*, 1999
46. **McGuire J, Pfeiffer NE, Weilke JM, Bartsch RC.** Failure of colostral transfer in calves dying from infectious disease. *Journal of the American Veterinary Medical Association* 169, 713-718, 1976
47. **McGuirk.** New approach to electrolyte therapy. *Cattle Practice* 6, 67-69, 1998
48. **Millemann Y.** Diagnosis of neonatal calf diarrhoea. *Revue de Medecine Veterinaire* 160(8-9), 404-409, 2009
49. **Parkinson T, Vermunt J, Malmo J.** Chapter 4: Diseases causing diarrhoea. *Diseases of Cattle in Australasia, A comprehensive textbook.* Pp 127-181. Wellington: Vetlearn: The New Zealand Veterinary Association Foundation for Continuing Education, 2010
50. **Parkinson T, Vermunt J, Malmo J.** Chapter 17: Calves: Management and Diseases. *Diseases of Cattle in Australasia, A comprehensive textbook.* Pp 627-659. Wellington: Vetlearn: The New Zealand Veterinary Association Foundation for Continuing Education, 2010
51. **Perez E, Noordhuizen J, Vanwuijkhuise LA, Stassen EN.** Management factors related to calf morbidity and mortality rates. *Livestock Production Science* 25(1-2), 79-93, 1990
52. **Roth BA, Barth K, Gygax L, Hillmann E.** Influence of artificial vs. mother-bonded rearing on sucking behaviour, health and weight gain in calves. *Applied Animal Science* 119, 143-150, 2009
53. **Schouten B.** Calf Rearing- Common Problems. *Proc*. *16th Annual Seminar, Society of Dairy Cattle Veterinarians NZVA* 16, 29-31, 1999
54. **Schouten B.** Calf and Heifer Rearing. *Proc. 20th Annual Seminar, Society of Dairy Cattle Veterinarians NZVA* 20, 101-106, 2003
55. **Schouten B.** Oral electrolytes in calves with diarrhoea. *Vetscript* 17(5), 28-30, 2004
56. **Schouten B.** Hypoglycaemia in newborn calves. *Vetscript,* 23(8), 31-33, 2010
57. **Schroeder B.** Sproule R, Saywell, D. Prevalence of rotavirus in dairy calves as diagnosed by ELISA. *Surveillance New Zealand* 12(2), 2-3, 1985
58. **Schuuman F.** Risk factors for mortality from diarrhoea in beef calves in Alberta. *Canadian Journal of Veterinary Research* 54, 336-372, 1990
59. **Silverlas C, Emanuelson U, de Verdier K, Bjorkman C.** Prevalence and associated management factors of Cryptosporidium shedding in 50 Swedish dairy herds. *Preventive Veterinary Medicine* 90(3-4), 242-253, 2009
60. **Sivula NJ, Ames TR, Marsh WE.** Management practices and risk factors for morbidity and mortality in Minnesota dairy heifer calves. *Preventive Veterinary Medicine* 27(3-4), 173-182, 1996
61. **Sivula NJ, Ames TR, Marsh WE, Werdin RE.** Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. *Preventive Veterinary Medicine* 27(3-4), 155-171, 1996
62. **Bush LJ, Staley TE.** Absorption of colostral immunoglobulins in newborn calves. *Journal of Dairy Science* 63, 672-680, 1980
63. **Svensson C, Hultgren J, Oltenacu PA.** Morbidity in 3-7-month-old dairy calves in south-western Sweden, and risk factors for diarrhoea and respiratory disease. *Preventive Veterinary Medicine* 74(2-3), 2006

64. **Svensson C, Lundborg K, Emanuelson U, Olsson SO.** Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. *Preventive Veterinary Medicine* 58(3-4), 179-197, 2003
65. **Thomas D, Stanton NL, Seville RS.** A Stable Eimerian Assemblage in Wyoming Ground Squirrels (*Spermophilus Elegans Elegans*)-Maintaining Viability Over Winter. *Journal of the Helminthological Society of Washington* 62(1), 1-5, 1995
66. **Trotz-Williams LA, Leslie KE, Peregrine AS.** Passive Immunity in Ontario Dairy Calves and Investigation of Its Association with Calf Management Practices. *Journal of Dairy Science* 91, 3840-3849, 2008
67. **Trotz-Williams LA, Martin SW, Leslie KE, Duffield T, Nydam DV, Peregrine AS.** Calf-level risk factors for neonatal diarrhea and shedding of Cryptosporidium parvum in Ontario dairy calves. *Preventive Veterinary Medicine* 82(1-2), 12-28, 2007
68. **Vasseur E, Rushen J. de Passille AM, Lefebvre D, Pellerin D.** An advisory tool to improve management practices affecting calf and heifer welfare on dairy farms. *Journal of Dairy Science* 93(9), 4414-4426, 2010
69. **Vermunt J, Stafford KJ, Thompson KJ.** Observations on colostral intake in newborn dairy calves. *New Zealand Veterinary Journal* 43(5), 205-206, 1995
70. **Vermunt J.** Calf rearing Part 1: Principles of calf rearingfrom birth to weaning. *Vetscript* 15(4), 4-6, 2002
71. **Vermunt J.** Calf rearing Part 2: The management of neonatal calf diarrhoea. *Vetscript* 15(5), 6-8, 2002
72. **Waltnertoews D, Martin SW, Meek AH.** Dairy calf management, morbidity and mortality in Ontario Holstein herds .3. Association of management with morbidity. *Preventive Veterinary Medicine* 4(2), 137-158, 1986
73. **Waltnertoews D, Martin SW, Meek AH.** Dairy calf management, morbidity and mortality in Ontario Holstein herds .4. Association of management with mortality. *Preventive Veterinary Medicine* 4(2), 159-17, 1986
74. **Wells SJ, Dargatz, DA, Ott SL.** Factors associated with mortality to 21 days of life in dairy heifers in the United States. *Preventive Veterinary Medicine* 29(1), 9-19, 1996
75. **Wesselink R, Stafford KJ, Mellor DJ, Todd S, Gregory NG.** Colostrum intake in dairy calves. *New Zealand Veterinary Journal* 47(1), 31-34, 1999
76. **Woods M.** Diseases and disease control in the weaned calf. Paper presented at the *Proc 16th Annual Seminar, Society of dairy cattle veterinarians NZVA,* Pp 131-141, 1999
77. **Younis EE, Ahmed AM, El-Khodery SA, Osman SA, El-Naker YFI.** Molecular screening and risk factors of enterotoxigenic *Escherichia coli* and *Salmonella spp.* in diarrheic neonatal calves in Egypt. *Research in Veterinary Science* 87(3), 373-379, 2009

Appendix 1

Descriptive statistics (continuous variables) from a study into calf morbidity and mortality risks due to diarrhoeic disease from 45 farms in the Waikato region of New Zealand

Continuous Independent Variables						
	Minimum	**1st Quartile**	**Median**	**Mean**	**3rd Quartile**	**Maximum**
Number of cows to calve	83	209	270	302	345	655
Number of heifers to calve	27	57	71	79	90	200
Number of replacements	25	60	80	85.2	100	200
Percentage replacements	13.0	19.6	22.7	22.8	25	35.3
Calf area (m^2)	0.4	1	1.5	1.6	2	4

Appendix 2

Descriptive statistics (categorical variables) from a study into calf morbidity and mortality risks due to diarrhoeic disease from 45 farms in the Waikato region of New Zealand

Categorical Independent Variables		**# of herds in group**
	Levels	Total # of herds=45
Breed code of herd (recat)	>50%Friesian	18 (40%)
	>50%Otherbreeds*	27 (60%)
New-born calves fed separately from older calves	Yes	42 (93.3%)
Colostrum warmed for first feed	Yes	28 (62.2%)
Colostrum stored in lidded drum	Yes	32 (71.1%)
Colostrum stirred	Yes	43 (95.6%)
Preservative added to colostrum	Yes	9 (20%)
What kind of preservative was added (recat)	None	36 (80%
	Commercial	8 (17.8%)
	Other**	1 (2.2%)
New calves fed from newly calved cows	Yes	42 (93.3%)
New calves trained to drink	Yes	44 (97.7%)
Calves fed colostrum from sick/induced cows and heifers	Yes	23 (51.1%)
Volume of colostrum fed to new calves (recat)	<=2litres	20 (44.4%)
	>2 litres***	25 (55.6%)
Are calves checked received enough colostrum	Yes	6 (13.3%)
How are calves checked have had enough colostrum	Visually	34 (75.6%)
	Measured	4 (8.9%)
	not checking	7 (15.6%)
Calves forcefed	Yes	6 (13.3%)
Time between calving and	1-6 hours and 6-12 hours	14 (31.1%)
first feed (recategorised)	12-24 and >24 hours****	31 (68.9%)
Colostrum supplied to Fonterra (milk company)	Yes	11 (24.4%)
Calves fed colostrum for first 4 days of life	Yes	43 (95.6%)
Frequency of feeding of calves	Once a day	4 (8.9%)
	Twice a day	38 (84.4%)
	>twice a day (incladlib)	3 (6.7%)
Time at which calves onto once a day feeding (recat)	Less than 3 weeks old	23 (51.1%)
	>3 weeks old*****	22 (48.9%)
Type of feeder initially used for youngest calves	gate rail	41 (91.1%)
	Calfeteria	3 (6.7%)
	Automated	1 (2.2%)
Age calves go onto calfeteria (recat)	<3 weeks	20 (44.4%)
	>3 weeks******	25 (55.6%)
Age calves go out to grass (recat)	>4 weeks old	19 (42.2%)
	<4 weeks old*******	26 (57.8%)
Same paddocks used every year	Yes	35 (77.8%)

Categorical Independent Variables		# of herds in group
	Levels	Total # of herds=45
Number calves per teat	1	43 (95.6%)
	1 to 5	2 (4.4%)
Number of teats changes as calves get older	Yes	22 (48.9%)
Number of teats increases or decreases as calves get older	Doesn't change	23 (51.1%)
	Increases	20 (44.4%)
	Decreases	2 (4.4%)
Supplement fed to calves	Yes	41 (91.1%)
Type of supplement fed	None fed	4 (8.9%)
	Concentrate	25 (55.5%)
	Hay and concentrate	13 (28.9%)
	Hay only or hay and silage	3 (6.7%)
Coccidiostat added to supplement feed	Yes	35 (77.8%)
Water offered in shed	Yes	32 (71.1%)
Frequency of changing water	Never changed/none offered	14 (31.1%)
	Daily/automatically refreshed	24 (53.3%)
	Every 2-3 days or >every 2-3 days	7 (15.6%)
Number of people rearing calves	One	15 (33.3%)
	Two	20 (44.4%)
	More than two	10 (22.2%)
All in all out system	Yes	9 (20%)
Number of calf sheds	1/2/3 or more	10/22/13
Bobby calves reared separately	Yes	13 (28.9%)
Calves moved between sheds	Yes	28 (62.2%)
Calves moved around the shed	Yes	40 (88.9%)
Calf shed >20m from cow shed	Yes	18 (40%)
Disinfectants used in calf sheds	Yes	30 (66.7%)
Disinfectant type used	None	15 (33.3%)
	Virkon	17 (37.8%)
	Other	13 (28.9%)
Timing of disinfectant use in calf sheds (recat)	None used	15 (33.3%)
	Betw seasons or during seasons	6 (13.3%)
	Both betw and during seasons********	24 (53.3%)
Calf feeders cleaned between uses	Yes	27 (60%)
Bedding replaced in the calf sheds	Yes	40 (88.9%)
Timing of bedding replacement in calf sheds (recat)	Not changed	5 (11.1%)
	Betw seasons or during season	19 (42.2%)
	Both betw and during seasons*********	21 (46.7%)
Bedding type used in calf sheds	Sawdust	14 (31.1%)
	Shavings	26 (57.8%)
	Woodchip	5 (11.1%)
Weaning weight of calves	60-80kg	8 (17.8%)
	80-100kg	37 (82.2%)

Weaning age of calves	6-8 weeks old	3 (6.7%)
	8-10 weeks old	18 (40%)
	10-12 weeks old	18 (40%)
	>12 weeks old	6 (13.3%)
Navel spray used	Yes	30 (66.7%)
Frequency calves picked up from paddocks per day	Once a day	40 (88.9%)
	Twice a day	5 (11.1%)
Timing from calving to first milking (recat)	<12 hours	11 (24.4%)
	>12 hours**********	34 (75.6%)
Maternal vaccination pre-calving (recat)	None	31 (68.9%)
	Rotavirus or Salmonella or both***********	14 (31.1%)
Treatment for calves with diarrhoea	None	3 (6.7%)
	Electrolytes	21 (46.7%)
	Antibiotics	2 (4.4%)
	Electrolytes and Antibiotics	19 (42.2%)
Sick calves isolated from healthy calves	Yes	42 (93.3%)
Sick calves isolated in a different shed	Yes	7 (15.5%)
Sick calves fed last	Yes	36 (80%)
Bought in animals quarantined	Yes/No/Not applicable	1 (2.2%)
	No	10 (22.2%)
	Not applicable	34 (75.6%)
Biosecurity measures applied in calf sheds	None	24 (53.3%)
	Gloves	7 (15.6%)
	Footbaths or washing boots	7 (15.6%)
	Gloves and footbaths or washing boots	7 (15.6%)

Footnotes

*breedcode was collapsed there were 4 Jersey herds and 23 Cross bred herds in the other breed category

**preservative types other included 1 farm which used formaldehyde and one farm which used yoghurt

***volume of colostrum fed was recategorised into over 2 litres and 2 litres or less, 2 farms fed 1 litre of colostrum and 18 farms fed 1.5 to 2 litres of colostrum

****time between calving and first feed was recategorised into 2 categories, category one included 5 farms where calves were fed at 1-6 and 9 farms where calves were fed at 6-12 hours and category two included 25 farms where calves were fed at 12-24 hours and 6 farms where calves were fed at >24 hours

*****time at which calves are put onto once a day feeding recategorised into less than 3 weeks (included 4 farms where calves are on once a day from start, 12 farms where calves were on once a day at less than 2 weeks old and 7 farms where calves were on once a day at 2-3 weeks old) and more than 3 weeks (included 11 farms where calves were on once a day at 3-4 weeks, 6 farms where calves on once a day at 4-5 weeks and 5 farms where calves were on once a day at 6-7 weeks)

******Age calves go onto calfeteria recategorised into less than 3 weeks (includes 2 farms from start, 11 farms from <2 weeks and 7 farms from 2-3 weeks) weeks and more than 3 (includes 9 farms from 3-4 weeks and 16 farms at >4 weeks)

*******Age calves go out to grass recategorised into <4 weeks (includes 6farms at <2 weeks, 4 farms at 2-3 weeks old and 16 farms at 3-4 weeks) and >4 weeks (includes 10 farms at 4-5 weeks and 9 farms at >5 weeks)

********Timing of disinfectant used in calf sheds recategorised so that category between or during season includes 3 farms using disinfectant between seasons and 3 farms using disinfectant during the season

*********Timing of bedding replacement in calf sheds recategorised so that category between or during season includes 15 farms replacing bedding between seasons and 4 farms replacing bedding during the season

**********Timing from calving to first milking recategorised into more than 12 hours (includes 2 farms at 1-6 hours and 9 farms at 6-12 hours) and less than 12 hours (includes 26 farms at 12-24 hours and 8 farms at >24 hours)

***********Maternal vaccination pre-calving recategorised into yes or no where yes includes 10 farms vaccinating for Rotavirus, 2 farms vaccinating for Salmonella and 2 vaccinating for both Rotavirus and Salmonella

Appendix 3

Recording sheets given to farmers at the beginning of the study

Calf Scour Recording Sheet

Diarrhoea is defined as faeces with a consistency looser than normally observed in calves, watery faeces occasionally with undigested milk particles or blood.

Please record all cases of diarrhoea in your calves even if you don't treat them.

				Please Tick	Please Tick	Please Tick
Date	**Calf number**	**Date of birth**	**Treatment**	**Bobby**	**Replacement**	**Died**

Appendix 4

Questionnaire completed by each farmer prior to the spring calving period

Appendix 5

Risk factor assessment sheet to be completed by clinician before the spring calving season

Risk Factors For Calf Scours

anexa animal health

1. Herd Size Information

a) Cows to calve ____
b) Heifers to calve ____
c) Replacements to rear ____

Comments: ____

2. Colostrum Management

a) Time from calving to first feed of new born calves
hours ____ or ____ days

b) Once a day or twice a day pick up of calves
once ____ or ____ twice

c) Observation of new calves and checking they get enough to drink ◯ Yes ◯ No

d) Time from calving to first milking
hours ____ or ____ days

e) Volume of colostrum fed ____ litres

f) Colostrum supplied to milk company
◯ Yes ◯ No

g) Colostrum warmed, stored in a lidded drum and stirred
◯ Yes ◯ No

h) Vaccination
◯ Yes ◯ No

Comments: ____

3. Calf Rearing Facilities

a) Number of calf sheds ____
b) Bobbies reared separately ____
c) Area per calf in metres² ____

Comments: ____

4. Calf Management

a) Type of feeder and age calves go onto calfeteria
Type ____ Age ____

b)	Feeder cleaned between use	◯ Yes	◯ No
c)	Disinfectants used in calf sheds	◯ Yes	◯ No
d)	Sick calves fed last	◯ Yes	◯ No
e)	Sick calves isolated	◯ Yes	◯ No
	if yes, in different shed	◯ Yes	◯ No
f)	Bedding replaced	◯ Yes	◯ No
g)	Bedding type		
h)	Same paddocks used every year for calves	◯ Yes	◯ No
i)	Biosecurity measures gloves, footbaths, washing boots	◯ Yes	◯ No
j)	Quarantine new calves bought in	◯ Yes	◯ No

Comments: ____