Why do some calves die and others thrive?

An investigation of risk factors impacting male calf health in Ontario

by

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ABSTRACT

WHY DO SOME CALVES DIE AND OTHERS THRIVE?
AN INVESTIGATION OF RISK FACTORS IMPACTING MALE CALF HEALTH IN ONTARIO

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This thesis is an investigation of the care of male calves on Canadian dairy farms, management factors on source dairy farms impacting mortality at veal farms, and clinical and metabolic factors impacting mortality after arrival at a veal facility. Finally, the diagnostic accuracy of bioluminescence in detecting contaminated colostrum feeding equipment was evaluated.

A cross-sectional survey was used to evaluate the care of male calves on Canadian dairy farms. Most respondents always fed colostrum and always fed male and female calves the same. However, a minority of respondents always navel-dipped or vaccinated male calves. The care of male calves differed greatly depending on geographical region, herd size and familiarity with the Code of Practice for the Care and Handling of Dairy Cattle.

There were several high-risk management practices impacting male calf health on veal farms identified through a cross-sectional study of source dairy farms sending male calves to two veal operations. The feeding method for colostrum, bedding used for male calves, veterinary involvement with calf health management, and the frequency of observation of the calving area were all associated with high mortality source dairy farms.

Many calves entered the milk-fed veal facility with a health abnormality and most of the mortality occurred in the first three weeks following arrival. Several risk factors were identified through the use of both a cohort and case-control study design, such as abnormal navel, dehydration, and body weight at arrival were associated with early and late mortality in the growing period. A lower level of immunoglobulin G and cholesterol in the serum was also associated with greater odds of early mortality.

Colostrum feeding equipment harbored a significant amount of bacterial contamination. Visual hygiene assessment was a poor indicator of bacterial count. The Hygiena™ AquaSnap and MircoSnap luminometry swabs were shown to be reliable predictors of total bacterial and total coliform counts, respectively.

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STATEMENT OF WORK DONE

Laboratory determination of blood parameters for Chapter 4 were performed by the Animal Health Laboratory, University of Guelph and the Saskatoon Colostrum Company.

Preparation of this thesis was completed by David Renaud in its entirety. This included study design, data collection, statistical analysis, interpretation, writing and preparation of manuscripts. Todd Duffield, David Kelton, Stephen LeBlanc and Derek Haley advised on study design, statistical analysis and provided edits on all chapters.

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95% CI: 95% confidence interval
ACC: aerobic colony count
AUC: area under the receiver operator characteristic curve
ATP: adenosine tri-phosphate
AS: AquaSnap Total swab
BRD: bovine respiratory disease
CC: coliform count
EU: European Union
FPT: failure of passive transfer
HMSF: High mortality source dairy farm
IgG: immunoglobulin G
IRR: Incidence rate ratio
LAB: lactic acid bacteria
LMSF: Low mortality source dairy farm
MS: MicroSnap Coliform swab
n: number of calves or samples tested
No.: number of calves
OR: odds ratio
P: p-value
PC: PRO-Clean swab
r: Spearman correlation coefficient
ROC: receiver operator characteristic
Ref.: Referent
RLU: relative light units
Se: sensitivity
Sp: specificity
SS: SuperSnap swab
TBC: total bacterial count
TCC: total coliform count
TP: serum total protein

CHAPTER 1: LITERATURE REVIEW
Challenges facing the Canadian veal industry

The Canadian veal industry is a small but critical agricultural sector. The premise of the industry is taking low value male calves of little genetic merit and creating a useable product as red meat. With the majority of pregnancies in the dairy industry resulting from the use of conventional semen (De Vries et al., 2008), roughly half of the pregnancies will result in male offspring leading to approximately 480,000 male calves being produced each year in Canada (Canadian Dairy Information Center, 2016). There are two main types of veal production in Canada; grain-fed and milk-fed. Milk-fed veal calves are predominately fed a milk-based diet and have a growing period of 20 weeks resulting in a finished live weight of 230 to 250 kg. Grain-fed veal calves are started on a milk-based diet and weaned onto a whole grain corn or grain supplement that they are fed for the remainder of the production period. The growing period is approximately 32 weeks resulting in a finished live weight of 320 kg. The veal industry is an important outlet for male calves from the dairy industry, however, depending on geographical location, some male calves will be filtered into the dairy beef sector. Dairy beef differs from veal in that the calves are castrated and slaughtered at a heavier weight and an older age. Ontario and Quebec are the largest producers of veal in the country producing over 200,000 slaughtered animals in 2015 (Agriculture and Agri-Food Canada, 2015).

Mortality, morbidity, animal welfare and antimicrobial resistance are major concerns that the industry currently faces. Precise mortality statistics are currently unavailable in Canada, however, Winder et al. (2016) presented a mortality risk of 7.6% over the entire production period at a single milk fed veal facility in Ontario. Industry experts suggest it may be higher with some outbreaks resulting in mortality spikes up to 15-20%. In Europe, mortality is lower with Pardon et al. (2012a) and Bahler et al. (2012) reporting a mortality risk of 5.3% and 3.6%, respectively. As mortality is a commonly used marker of animal welfare (Ortiz-Pelaez et al., 2008), there needs to be greater emphasis on its prevention.

Morbidity levels (crudely defined by antimicrobial use) in Canada are currently unknown. Sargeant et al. (1994) last evaluated antimicrobial use in milk-fed veal raised in Ontario, showing that 59% of calves received at least one treatment day of individual medication, with all calves receiving group oral antibiotics in the first week of production. The

level of individual antibiotic treatment is likely lower in today's production system in Canada; however, group oral antibiotic therapy on arrival remains a standard protocol in both Canada and Europe (Jarrige et al., 2017; Pardon et al., 2012b). The high level of antimicrobial use in the veal calf sector (Bos et al., 2013) has been associated with the development of antimicrobial resistance in commensal and animal pathogens (Catry et al., 2016; Di Labio et al., 2007). With little evidence available to support the use of oral group antimicrobial therapy, it would be judicious to explore the necessity and effectiveness of this strategy (Jarrige et al., 2017) as it represents the majority of antimicrobial consumption (Lava et al., 2016; Jarrige et al., 2017; Pardon et al., 2012b).

Animal welfare concerns are a continually pressing issue and not solely related to challenges with morbidity and mortality. Housing represents a significant issue, with some milk-fed veal producers still utilizing individual housing for the entire production period in Canada. Clearly, the inability to freely move is a significant issue but will be addressed with the publication of the Code of Practice for the Care and Handling of Veal Cattle in the fall of 2017. Anemia (Wilson et al., 1994), low volumes of milk replacer fed to calves in grain-fed veal, slatted flooring, abomasal lesions (Bahler et al., 2010) and lack of access to bedding, are other challenges facing the veal industry and were reviewed by the scientific committee for the Code of Practice for the Care and Handling of Veal Cattle (de Passillé et al., 2016).

For the veal industry to remain economically viable, it is imperative to explore and address these challenges, which begin on the dairy farm and continue once the calves reach the veal farm. In this literature review, the challenges associated with mortality and morbidity will be explored through an investigation of risk factors impacting them.

Management of male calves on dairy farms

Calves in the veal industry have the highest level of mortality in the first few weeks of calf rearing (Bahler et al., 2012; Pardon et al., 2012a; Winder et al., 2016), which is similar to the distribution of mortality occurring in female calves on dairy farms (Waltner-Toews et al., 1986a; Wells et al., 1996; Windeyer et al., 2014). Colostrum management (Donovan et al., 1998; Wells et al., 1996; Windeyer et al., 2014), calving management (Nielson et al., 2002; Waltner-Toews et al., 1988c), housing (Svennsson et al., 2006; Waltner-Toews et al., 1988b; Windeyer et al., 2014) and, calf nutrition (Waltner-Toews et al., 1988b) have all been associated with increased

mortality and morbidity in female dairy calves in large observational studies demonstrating that neonatal calf care is critical for the management of female calves (Gulliksen et al., 2009; Wells et al., 1996; Windeyer et al., 2014). Despite significant differences in the management of veal and dairy calf rearing, these management areas likely have significant impacts on calf health once they reach the veal industry and thus will be explored as potential factors that impact male calf health. As there is truly a lack of peer-reviewed literature tracing male calves back to source dairy farms, this is an area that requires further exploration.

Calving Management

Parturition can be a challenging event for both the cow and the calf (Murray and Leslie, 2013). Dystocia, defined as calving difficulty resulting from prolonged spontaneous calving or prolonged assisted extraction (Mee, 2004), occurs more often in male calves than female calves (Olson et al., 2009; Johanson and Berger, 2003) likely due to male calves being larger and heavier than female calves at birth (Johanson and Berger, 2003). Dystocia can cause internal injuries such as bleeding and fractures, impaired breathing, inadequate supply of oxygen and an impaired ability to thermoregulate, creating a calf with poor vitality (Murray and Leslie, 2013). Higher perinatal mortality, lower rates of successful passive transfer of immunity, and higher morbidity and mortality in the pre-weaning period (Barrier et al., 2013; Eaglen et al., 2011; Lombard et al., 2007) are consequences that can arise from dystocia. With 30 to 40% (Lombard et al., 2007; Olson et al., 2009) of calvings involving male calves requiring some type of assistance at birth, proper management of the calving process could be critical to improving the health and welfare of male calves. Regular surveillance during the second stage of calving, combined with timely intervention, will prevent dystocia as long as intervention is based on appropriate decisions on when to intervene, how to intervene and when to solicit veterinary advice (Mee, 2004). Genetic management through sire selection, body weight at insemination and age, body weight and body condition score at calving are also factors that could be explored to reduce the incidence of dystocia (Mee, 2004). The use of non-steroidal anti-inflammatory drugs following calving is a potential therapy to provide to male calves when dystocia occurs (Murray et al., 2016), however, this strategy may be cost-prohibitive for some dairy producers to implement.

Colostrum Management

Colostrum management is a vital component of male calf management. Some studies have highlighted the importance of passive transfer in male calves at arrival with lower levels of respiratory disease (Pardon et al., 2015), morbidity (Davidson et al., 1981; Stillwell and Carvalho, 2011; Wilson et al., 2000), mortality (Davidson et al., 1981; Stillwell and Carvalho, 2011) and higher average daily gains (Pardon et al., 2015) being seen if the calves did not have failure of passive transfer (FPT). However, there is difficulty in comparing the results as each study evaluated passive transfer differently, including the use of total protein, IgG levels and a commercial quick IgG test. Another challenge clouding the interpretation of the results is that the age of the calves at arrival at the veal facilities are different in all of the studies. Pardon et al. (2015) conducted their study in Belgium where calves arrive at the veal facilities at an older age compared to North America where calves are sent to the male calf raisers in the first days of life (Stillwell and Carvalho, 2011; Wilson et al., 2000). Despite the known importance of colostrum in male calves, 38% (<5.5g/L serum total protein (TP) (Wilson et al., 2000) to 41% (<5.2g/L serum TP (Trotz-Williams et al., 2008) and <10g/L IgG (Pardon et al., 2015)) of calves destined for veal production have FPT. These levels of FPT impact the health and welfare of male calves, however, similar FPT levels are found in female calves (Trotz-Williams et al., 2008). Achieving early and adequate intake of high quality colostrum will aid in reducing the levels of FPT (Godden, 2008). While progress has been made, colostrum management practices still need to improve to ensure all calves have adequate passive transfer.

Nutrition

The importance of early life nutrition in dairy calves has been well documented. Nutritional programs providing higher volumes of milk increase weight gain, and may aid in improving disease resistance over the pre-weaning period in both male and female dairy calves (Todd et al., 2017; Khan et al., 2007; Ollivett et al. 2012). Williams et al. (1981) also demonstrated that feeding lower levels of milk replacer lead to higher levels of mortality in male veal calves. As a lower weight at arrival to a veal facility has been associated with greater risk of mortality (Winder et al., 2016), nutrition on the source dairy farm and its influence on average daily gain and immune function might be important factors in prevention of morbidity and mortality in male calves.

The source of milk being fed to calves may also influence male calf health. Non-saleable whole milk or "waste" milk represents a low-cost source of nutrition commonly provided to calves in Canada and the United States (USDA, 2010; Vasseur et al., 2010). However, the inconsistent composition (Hill et al., 2009), high levels of bacterial contamination and antibiotic residues (Moore et al., 2009; Selim and Cullor, 1997) seen in waste milk may impact the health and performance of calves and lead to development of antibiotic resistance (Aust et al., 2012; Maynou et al., 2017). Pasteurization achieved by heating the milk to 63°C for 30 minutes or 72°C for 15 seconds inactivates bacteria, most viruses and protozoa (Aust et al., 2012). When this process is completed with waste milk, some of the negative implications are dissipated specifically with better growth and health (Godden et al., 2005; Jamaluddin et al., 1996); however, antibiotic residues within the milk are not affected (Aust et al., 2012). With male bob calves having higher levels of antibiotic residues at slaughter compared to other bovine categories (OMAFRA, 2015; USDA, 2017), this may be a potential source for violative residues but also in the development of the high levels of antimicrobial resistance currently present in veal calves (Catry et al., 2016; Di Labio et al., 2007).

Housing

Housing has also been implicated as a factor influencing calf health. Housing calves individually provides some health advantages to group pens, likely due to greater exposure to infectious agents in group housing, which leads to higher levels of morbidity and mortality (Gulliksen et al., 2009; Svensson et al., 2006; Waltner-Toews et al., 1986b). However, group housing allows for more social interaction and increased physical exercise (Vasseur et al., 2010). Pair housing calves may provide an intermediary step where calves can experience social behavior without compromising health (Jensen and Larsen, 2014) and may also increase the performance of the calves (Costa et al., 2015; Vieira et al., 2010).

Proper ventilation is critical for the prevention of bovine respiratory disease (BRD) in pre-weaned dairy heifer calves (Nordlund, 2008; Windeyer et al., 2014). With pneumonia being one of the most common causes of mortality and morbidity on veal operations (Pardon et al., 2012a), ventilation at the source dairy farm may influence its incidence in male calves. Bedding has also been shown to influence BRD. An increased provision of long straw for nesting reduced the prevalence of BRD (Lago et al., 2006). Panivivat et al. (2004) also demonstrated advantages

to long straw through lower number of scour days and lower coliform counts when compared to other bedding types. The challenge with the interpretation of the studies conducted on bedding is the low number of calves/barns within each group and the studies being conducted solely during specific times of the year. Ultimately, what remains unclear is if housing of male calves on source dairy farms has an impact on health as this has not been studied. Further understanding of ventilation and bedding is also required in dairy heifer calves as well.

Navel Antisepsis

Navel antisepsis has long been recommended for the care of the navel for newborn calves (Hadley, 1954), and is still recommended today by industry experts (Wieland et al., 2017). However, there is a paucity of literature that demonstrates the effectiveness of navel antisepsis in preventing local or systemic illness. Only one study, described in an abstract, used a negative control group and concluded "this is the first controlled study that demonstrate the effectiveness of a navel dip" (Grover and Godden, 2011). However, their use of historical controls for the negative control group leaves doubt as to the effectiveness of navel antisepsis. The remainder of the published studies utilize a positive control group with 7% iodine tincture, the most commonly used navel antiseptic (Robinson et al., 2015; Weiland et al., 2017). Both studies concluded that there were no differences between the types of navel dips utilized. As two epidemiological studies have reported an increase in disease with the use of navel antisepsis (Waltner-Toews et al., 1986c; Windeyer et al., 2014), there is a real need to prospectively test navel antisepsis against a negative control group.

Differences in management between male and female calves

It has long been speculated that dairy producers are treating male calves with some level of neglect (Bahler et al., 2012; Schnepper, 2001); however, few studies have been conducted to confirm this theory. Fecteau et al. (2002) and Shively et al. (2016) found male calves were more likely to be fed contaminated colostrum, receive a smaller volume of colostrum, have delayed colostrum feeding, or be left with the dam as a mechanism to feed colostrum, suggesting some discrimination exists in regard to colostrum management. Clearly, these differences may impact the health and welfare of male calves (Pardon et al., 2015), however, the extent to which these

differences in colostrum management exist in Canada, and if similar differences exist with respect to other management practices, such as nutrition or housing, is unclear.

Transportation of young calves

Transportation can be a severe stress for animals (Trunkfield and Broom, 1990). The challenges faced by young male calves in the context of transportation are handling, removal from familiar conditions, mixing with unfamiliar animals often crowded with fluctuating temperatures and deprivation from food and water (Trunkfield and Bloom, 1990). These stresses can act as an immunosuppressant and create a less effective immune system increasing disease in transported calves. As all calves in the current context of the industry must be transported, there are several factors impacting the influence of transportation which are age, length and season at transport.

Age at Transport

Knowles (1995) explored the impact that age at transport has on mortality in the post-transport period, and suggested a minimum age at transport of 4 weeks of age would be adequate to reduce the negative impact that transportation has on calf survival. Many studies utilize Knowles (1995) as a reference to describe the inverse relationship between age at transport and mortality; however, the evidence provided by these studies is insufficient to reach this conclusion. Staples and Haugse (1974) found higher mortality in calves transported in the first two weeks of life compared to older calves; however, the data were collected through a survey and were likely based on an estimate of age at transport for the purchased calves. Barnes et al. (1975) found that mortality was highest in calves transported at 2 and 3 days old compared to three or four days but this was a conference abstract with no statistical analysis presented and the authors mentioned the study was not designed to evaluate calf survival. It was also suggested by a cited article in the Knowles (1995) review that mortality rates were higher in transported calves than un-transported calves (Leach et al., 1968) but this study solely evaluated mortality in calves on home-bred farms compared to purchased calves, and did not have a transportation component. As it is unclear whether the association with mortality is due to increased disease pressure occurring in the first weeks of life (Wells et al., 1997) or due to transportation, further studies

need to be conducted comparing those transported at various ages and those retained at the source farm to truly evaluate the effect age at transport has on morbidity and mortality.

The hematological and behavioural response of young calves to transport is different from that of older cattle and calves. Most studies show that calves less than 4 weeks of age show little elevation in cortisol (Fell and Shutt, 1986; Mormede et al., 1982) or change in heart rate (Knowles et al., 1995) when compared to older cattle. However, as there is low reactivity of the adrenals to adrenocorticotropic hormone in young calves (Hartmann et al., 1973), it suggests that young calves may not be able to respond to transport stress (Knowles et al., 1995; Mormede et al., 1982) and the lack of hematological changes in younger calves does not signify that they are more capable of handling transportation. Behaviourally, younger calves tend to lie down more during transit when compared to older calves, but less when compared to a non-transported cohort (Grigor et al., 2001; Jongman and Butler, 2014). However, in the transported group, younger calves laid down more often during the pre- and post- transport period compared to older animals, indicating that physiology and lying behavior may be fundamentally different depending on age (Jongman and Butler, 2014). Hence, younger calves, both during and immediately following transit, should be provided with comfortable bedding material to ease the burden of transportation. Straw bedding was proposed as an ideal bedding substrate for transportation by Jongman and Butler (2014) as it increased lying time both during transit and at arrival.

Even if an appropriate age at transportation was identified, it would be extremely difficult to verify the age at which the calves were transported. Cord dryness has been used to estimate age at arrival in young calves (Wilson et al., 2000), but was shown to be a poor indicator of age (Hides and Hannah, 2005). The ability of the calf to move without significant intervention by the handler has also been proposed as a mechanism to gauge the age of a group of calves. Younger calves were found to be more difficult to move, and to take more interventions by the handler to move the calves (Jongman and Butler,2013); however, it was concluded that the calves are easy to move regardless of age. Hence, practically it would be very difficult to enforce an age at transport without the presence of a system for age verification that included trace-back to the dairy farm of origin.

Length of Transport

The length of time that young calves are in transit has an impact on their health and welfare. As time in transit for week old calves increases, mortality occurring during transport increases. This may reflect transport design, continuous motion and/or lack of rest as suggested by Cave et al. (2005). Other studies have shown that there are hematological changes that occur with increasing duration of transportation. Creatine kinase, a marker of muscle damage, increases when calves are transported, and remains elevated for several days following transport, highlighting that transportation causes wear and tear on the calf (Fischer et al., 2014; Knowles et al., 1999). A higher level of hypoglycemia and mobilization of body reserves was observed in calves transported long distances, likely due to the effect of food deprivation and an increased energy demand induced by transport and manipulations (Knowles et al., 1997; Mormede et al., 1982). These changes remained for nearly a week following transport, however, the growth performance of the calves was not affected by journey length (Mormede et al., 1982). Dehydration and an increased incidence of respiratory disease is also seen with long-lasting transport (Knowles et al., 1997; Mormede et al., 1982). In order to reduce the level of dehydration and mobilization of energy reserves, Knowles et al. (1997; 1999) fed calves during transit and it was shown to alleviate some of these effects of prolonged transit. However, the benefits were minor and this strategy may not be practical to implement commercially. Despite the evidence provided by the studies evaluating the hematological changes with prolonged transport, the findings should be interpreted with caution given the small number of calves, and the older ages of the calves used for transport study.

Season of Transportation

Young calves are very prone to thermal stress (Roland et al., 2016); particularly calves that experienced dystocia (Vermorel et al., 1989). Hence, during transportation it is important to mitigate factors associated with climatic changes and extremes. Winter transport may influence calf health more so than other seasons, with mortality following transport reported to be highest in the winter (Staples and Haugse, 1974; Winder et al., 2016). Changes in body weight and rectal temperature are also more severe during winter transport (Knowles et al., 1997; Knowles et al., 1999). To improve the calves' tolerance of sustained cold, it is important to ensure that the

hair coat and bedding is dry, lying areas are protected from drafts and sufficient nutritional energy is provided (Roland et al., 2016). Pre-transport nutrition may also be important, as restrictive feeding can have a negative effect on young calves, as they deplete body fat stores quickly (Roland et al., 2016). Clearly, more research needs to be done to understand the impact of cold weather transport, but with the winter season being shown to be the season of highest mortality on veal farms (Winder et al., 2016), mitigating these environmental conditions could be part of the overall strategy to improve male calf health and welfare.

Health status and treatment at arrival to a veal facility

Male calves face many challenges prior to arrival at a veal facility. Many of the factors have been previously discussed in the context of transportation, and management on the initial source dairy farms. As previously mentioned, most of the mortality occurs in the first few weeks following arrival, suggesting that calves which have started to move along the health disease continuum are entering the veal facility.

Health abnormalities

Wilson et al. (2000) was the first publication to examine calves for health abnormalities at arrival at a veal facility. Using a standardized health scoring physical examination, many calves were identified with health abnormalities. The most concerning findings were, of the calves examined at arrival, 28% had low body condition score, 26% were dehydrated, 32% had abnormal navels, 17% had diarrhea and 8% were in very poor condition. There were some associations identified between health abnormalities and medical treatment; more desirable navels having more treatments for respiratory disease and less treatment for other disease (navel, joint, eye and ear impairments), whereas calves with respiratory abnormalities and diarrhea at arrival received more treatment. However, multiple veal facilities were used in this study and the varying treatment protocols, and the variability among personnel making treatment decisions were not controlled for in the analysis. The associations identified in the study were based on univariable analyses, without accounting for covariates and potential confounders. Also, when selecting the calves in groups of greater than 100, only 50% were sampled, but no mention of random selection was provided, potentially creating selection bias. Non-differential misclassification bias may have also occurred as no mention of training for the several

individuals whom scored the calves was provided. Thus, the associations with medical treatment should be interpreted with caution.

Bahler et al. (2012) also evaluated calves at arrival at veal facilities and utilized a simplified subjective scoring system when compared to Wilson et al. (2000). The investigators had similar findings to Wilson et al. (2000), with 23% and 16% having diarrhea and an abnormal navel, respectively. The major difference was that 49% had respiratory symptoms at arrival, which is likely a reflection of the calves arriving at the veal facilities at a much older age. In multivariable logistic regression analysis, male gender, the number of antimicrobial treatments, and insufficient wind deflection were associated with mortality during the production period. The male gender may be related to what has been previously discussed in regard to discriminatory treatment at the farm of origin, however, the higher use of antibiotics being associated with mortality is an interesting finding. Higher levels of antibiotics are related often to outbreaks of disease but with the general condition of the calf being related to mortality in univariable analysis, potentially antibiotic treatment may be an intervening variable. The ability to extrapolate these study findings to a North American context is difficult as the calves are much older at arrival, many were dairy producers' raising veal calves, and automated calf feeders were predominantly used. These factors are extremely rare in both milk and grain-fed veal in Canada and the United States.

Pempeck et al. (2017) recently evaluated calves at arrival to veal facilities in Ohio. Again, the findings were very similar to the previous 2 studies with 35% dehydrated, 14% diarrhea, and 27% inflamed navels. However, in agreement with Wilson et al. (2000), few respiratory symptoms were found in the calves. The authors attempted to create a logistic regression model to evaluate the effects of health parameters at arrival with early mortality, however, the low number of calves examined limited the power of the study.

Ultimately, these studies provide clear evidence that a diseased population of animals are entering the veal industry. However, what is unclear is the impact health status at arrival has in terms of morbidity, mortality and growth performance.

Treatments administered at arrival

With the relatively large number of calves entering the veal industry with health abnormalities, it is not a surprise that a commonly used management strategy is to provide

antibiotics in the milk replacer to all calves for the initial portion of the growing period (Pardon et al., 2012). However, as previously mentioned, there is little evidence to support this universal approach as a prudent strategy. Berge et al. (2005) randomly assigned young calves purchased from multiple dairy farms to receive in-milk antibiotic treatment or no oral antibiotic treatment beginning at arrival. In the study, calves receiving in-milk antibiotic treatment had greater weight gain and lower morbidity compared to calves not receiving antibiotics within feed. However, there were many limitations to this study. Antibiotics were provided for the duration of the growing period, which is now illegal in the United States and in the European Union (Smith, 2013), and is not common practice on veal farms. Morbidity was defined as days to first treatment and the authors did not describe the total quantity of antibiotics provided to the groups, which is likely less in the group of calves not provided with antibiotics in the milk. During the first 28 days following arrival, 18% of calves died, which is likely a reflection of an outbreak of Salmonella and fluctuating weather conditions experienced during the study period. The calves were also limit fed milk (1.86 L provided twice daily), which is not common practice in milk-fed veal. Another study used to support the in-milk antibiotics approach was conducted by Rerat et al. (2012). The study used in-milk antibiotics for seven days, a positive control group (injectable tulathromycin once subcutaneously), and a negative control group, to detect differences in morbidity and average daily gain at a veal facility in Switzerland. The negative control group had a higher incidence rate of respiratory disorders and lower average daily gain compared to the other groups, however, average number of treatment days per calf were identical. There are some limitations to this study. The analysis was conducted on 3 groups of 20 calves that were housed in 3 totally separate barns with no evidence of randomization being used for entry into the groups. This design could seriously compromise the findings of the study as potentially different environmental conditions could be present in the barns such as ventilation but also with only 3 groups of calves evaluated, there is a great chance of a type 1 error occurring. This study also does not reflect the typical North American veal facilities as calves arrived at a much older age at the veal farm.

Contrasting results to the above studies were presented by Berge et al. (2009), where calves receiving antimicrobials in-feed had a greater risk of diarrhea compared to calves not fed antimicrobials. This result is similar to other studies (Shull and Frederick, 1978; Rollin et al., 1986) and is most likely caused by a disturbance of the commensal enteric flora. However, this

study is not reflective of typical veal production as it was completed at a single dairy farm where calves did not undergo commingling or transportation. As the addition of antibiotics to milk and milk replacers is being strongly discouraged worldwide (Smith, 2013), it is critical to develop alternatives for the prevention and control of disease in calves.

The addition of probiotics may provide an alternative to antibiotics for the prevention of calf diarrhea. Donovan et al. (2002) conducted a non-inferiority trial with a probiotic and a commonly used antibiotic fed to calves during the pre-weaning period. The author concluded that there were no differences in the overall performance or health of the calves when fed probiotics or antibiotics. Two meta-analyses were conducted to evaluate the efficacy of lactic acid bacteria (LAB), a commonly used probiotic in calves. It was concluded that the risk of diarrhea in calves treated with LAB was significantly lower compared to negative controls. However, the beneficial effect was seen only in calves that were fed whole milk (Signorini et al., 2012). If milk replacer was used, LAB had no effect on improving health of the calves (Signorini et al., 2012) but a higher average daily gain was reported (Frizzo et al., 2011). Another group of probiotics, *Faecalibacterium prausnitzii*, has also been shown to have promise given as an oral bolus in both the first and second week of life. The group bolused with the probiotic had significantly lower incidence of severe diarrhea, lower morality rate associated with severe diarrhea and gained more during the pre-weaning period when compared to negative controls (Foditsch et al., 2015). With the amount of evidence available to support the use of probiotics, the veal industry should consider switching away from in-milk antibiotics and replace with the addition of probiotics. However, there need to be more studies conducted at veal facilities to truly confirm this difference, as male calves experience a significant health challenge prior to arrival.

As pneumonia is a common cause of morbidity and mortality on veal farms (Pardon et al., 2012), the administration of intranasal vaccines against common respiratory pathogens is another common procedure that is administered at arrival, or within the initial growing period. Mucosal vaccination has an advantage over systemic vaccines, as young calves are able to mount a mucosal immune response in the face of maternal antibodies (Hill et al., 2012). Information is again limited when evaluating the literature in the veal context. A randomized clinical trial was conducted in Italy evaluating the effect of a viral intranasal given at 12 days following arrival (Cavirani et al., 2016). The study found a significant reduction in lung lesion score and daily

antibiotic treatments when utilizing simple statistical analysis. These findings are similar to what was reported by Ollivett et al. (2012), in a clinical trial on dairy heifer calves. The lack of evidence available in veal calves makes the recommendation of blanket intranasal vaccination difficult but it could be another alternative to curb antibiotic use in veal calves.

Rationale for thesis

The focus of this thesis is on male calf health. There has been very little literature published in North America on this topic in the previous two decades. With animal welfare, as well as antimicrobial resistance and use becoming increasingly important, a better understanding of factors impacting male calf health is needed to address these challenges.

Thesis objectives

The specific objectives of each chapter were:

Chapter Two

- Describe management practices associated with the early rearing of male calves on Canadian dairy farms

Chapter Three

- Assess the association of calf management practices on source dairy farms with mortality occurring on veal farms

Chapter Four

- Describe the health status of calves at arrival to a veal facility and to associate characteristics of the arriving calf with early and late mortality

Chapter Five

- Identify clinical and metabolic factors associated with mortality occurring in the first 21 days following arrival at a veal facility

Chapter Six

- Validate the Hygiena^{TM} AquaSnap Total (AS), SuperSnap (SS), Pro-Clean (PC) and MicroSnap Coliform (MS) swabs, as well as visual hygiene assessment, to detect elevated bacterial counts in colostrum feeding equipment

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CHAPTER 2: MANANGEMENT PRATICES FOR MALE CALVES ON CANADIAN DAIRY FARMS

As previously published

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MANANGEMENT PRATICES FOR MALE CALVES ON CANADIAN DAIRY FARMS

ABSTRACT

Morbidity, mortality, and antimicrobial use and resistance are major concerns in the rearing of male dairy calves, so information to support disease prevention is important. The objective of this cross-sectional study was to describe management practices associated with the care of male calves during their first days of life on Canadian dairy farms. A survey was completed by dairy producers across Canada from March 1 to April 30, 2015. The survey had 192 questions covering producer background, farm characteristics, biosecurity practices, disease prevalence, calf health, animal welfare, lameness, milking hygiene, reproduction and internet/social media use. A total of 1,025 surveys were completed online, by telephone, or by mail, representing 9% of all dairy farms in Canada. Five percent of respondents (n=49) answered that they had euthanized at least one male calf at birth in the previous year and blunt force trauma was used commonly in these cases. The majority of respondents always fed colostrum to male calves, however 9% (n=80) did not always feed colostrum. Nearly 40% (n=418) of respondents reported always dipping the navels of male calves, 12% (n=123) vaccinated male calves and 17% (n=180) did not provide the same quantity of feed to male calves as heifer calves. The care of male calves differed greatly depending on geographical region of the respondents. However, some regional effects may be confounded by economic conditions and the logistics of marketing male dairy calves in different parts of the country. Herd size was another important variable in many aspects of the management of male calves on dairy farms. Larger herd sizes were more likely to use an appropriate method of euthanasia at birth, but were less likely to always feed colostrum to their male calves, or feed them the same as female calves. Familiarity with the Code of Practice for the Care and Handling of Dairy Cattle by respondents was associated with better care of male calves on dairy farms. The results of this survey suggest that there is variable treatment of male dairy calves on Canadian dairy farms and that there are opportunities to improve health management of male calves on the farms of origin.

Key Words: Male calf, management, welfare

INTRODUCTION

Male calf health and welfare continue to be lingering issues in the dairy industry worldwide. New Zealand and Australia do not have well-established industries for raising male dairy calves leading to the majority being transported long distances to be slaughtered within days of birth (Cave et al., 2005). Due to effects of distance travelled and environmental stressors, male calves en route to slaughter plants experience high levels of mortality during transit (Cave et al., 2005). In Europe and North America, the majority of male dairy calves contribute to the red meat industry. The EU has specifically addressed a significant number of public concerns regarding animal welfare through the implementation of strict animal housing and nutrition requirements for male calves being raised for meat production (Council Directive, 2008). The high level of importance placed on male calf welfare in the EU has helped to address some public criticism; however high levels of antimicrobial use and resistance have become major concerns for the veal industry (Pardon et al., 2014). In North America, there has been little published research on male calf health and welfare over the past two decades. As antimicrobial resistance, mortality, and morbidity remain high among male dairy calf industries (Cook et al., 2011; Pardon et al., 2012; Winder et al., 2016), an increased focus on disease prevention needs to be a priority.

In heifer calves, many studies have highlighted the importance of neonatal calf management, on both the short and long term survival of these calves (Weaver et al., 2000; Lombard et al., 2007; Windeyer et al., 2014). The highest risk for mortality occurs in the first 21 days following arrival to male calf housing, (Pardon et al., 2012; Winder et al., 2016) suggesting that calf management on dairy farms also plays a key role in the prevention of mortality in male dairy calves.

Providing a sufficient quantity of good quality colostrum to newborn calves is an integral component of male calf management because failure of passive transfer (FPT) in male calves is associated with an increased risk for many diseases (Postema and Mol, 1984; Pardon, 2015). Despite the known importance of feeding colostrum, FPT is estimated to be common among male calves (Wilson et al., 2000; Schnepper, 2001; Pardon et al., 2015). It is interesting to note that a relatively recent study evaluating FPT (Trotz-Williams et al., 2008), found there was no difference in FPT between male and female calves, suggesting that poor colostrum management is widespread.

Another management practice that is used to increase host resistance to disease is the administration of vaccines. In young calves (3-8 days of age), the administration of an intra-nasal modified live vaccine against major viral pathogens of the bovine respiratory disease (BRD) complex has been shown to have a significant disease sparing effect, reducing clinical signs and pulmonary lesions (Xue et al., 2010). Despite the short duration of immunity induced by intranasal vaccination (Ellis et al., 2013), these vaccines may have utility in male calves raised for veal or dairy beef, as pneumonia has been found to be the main reason for antimicrobial use and mortality in veal operations (Pardon et al., 2012; Lava et al., 2016).

Early life nutrition also plays a role in increasing immune function and disease resistance. Malnourished calves have higher concentrations of blood cortisol, impaired lymphocyte function (Drackley, 2005) and take longer to recover from the effects of a *Cryptosporidium parvum* infection (Ollivett et al., 2012). Because male calves are often subjected to long transit times to their rearing site during which they may experience cold or heat stress, early adequate nutrition is critical for their survival (Roland et al., 2016).

The objective of this study was to describe management practices associated with the early rearing of male calves on Canadian dairy farms.

MATERIALS AND METHODS

Experimental Design

A national cross-sectional study (Bauman et al., 2017) was conducted between March 1 and April 30, 2015 to collect data on management practices on Canadian dairy farms. A comprehensive questionnaire was developed by representatives from four veterinary schools and questions were created to address key management and disease priorities (Bauman et al., 2016). These questions were then modified based on questions from other national surveys (USDA, 2007) and in consultation with other Canadian dairy researchers. An advisory group was created and consisted of dairy producers, government representatives and veterinarians to provide feedback with regards to the survey content. The final questionnaire consisted of 192 questions which were subdivided into producer background information, farm characteristics, biosecurity practices, disease prevalence, calf health, animal welfare, lameness, milking hygiene, reproduction and internet/social media use.

Human ethics approval was received from each participating university: University of Calgary (REB#14-2481), University of Guelph (REB#14DC025), Université de Montréal (15-

007-CERES-D), and the University of PEI (REB#6006095). The questionnaire was available in 3 formats, an on-line platform (Qualtrics[TM] (https://www.qualtrics.com/)), a Word document for mailing out or as a script to allow administration over the telephone. To optimize the response rate, an incentive ($20 gift card) was provided to the first 250 respondents.

Respondents were recruited through a letter of invitation that was mailed to every licensed dairy producer in Canada. The producer contact information was obtained through provincial milk marketing boards. To ensure that confidentiality was maintained, a unique anonymous code was assigned to each producer by the marketing board. The letter of invitation outlined the study scope and presented the options to complete the survey. The methods made available were the website address for online completion of the questionnaire, a quick response code linked to the website, a toll-free number with voicemail where requests could be made for completion over the telephone or on paper. In addition, a reply post card with postage paid that contained the producer's unique code and contact information could be used to notify the researcher that they wished to have a paper version of the questionnaire mailed to them. In addition to the data obtained through the producer completion of the questionnaire, milk production and farm demographic data were obtained for every Canadian dairy farm from the respective provincial marketing board. A more thorough description of the questionnaire design is presented by Baumann (2017).

Statistical Analysis

All statistical analyses were conducted using Stata 14 (StataCorp, 2015). Data were imported from Microsoft Excel into Stata 14 and checked for completeness. A causal diagram was created to evaluate the relationships between the potential exploratory variables and the outcomes of interest (Figure 2.1). Descriptive statistics were generated on all explanatory variables in the dataset.

Six explanatory logistic regression models were created to evaluate the outcomes 1) euthanasia of male calves at birth, 2) method of male calf euthanasia at birth, 3) colostrum feeding of male calves, 4) navel dipping of male calves, 5) vaccination of male calves and 6) feeding of male calves. The model assumption of linearity of continuous variables was assessed by plotting the logarithmic odds of the outcome against the variable. If a variable failed to meet the linearity assumption, the variable was categorized. Herd size did not meet the linearity assumption in any of the logistic models and was re-categorized based on quartiles with the first,

second, third and fourth quartile being 10-38, 39-54, 55-87 and 88-888 lactating cows, respectively. Co-linearity among the explanatory variables was tested using Spearman rank coefficients. If the correlation coefficient between 2 variables was ≥ 0.7, only one variable was retained based on fewest missing values, reliability of measurement and/or biological plausibility. Univariable logisitic regression models were constructed to screen for variables that were unconditionally associated with the outcome using a liberal P-value (P) of 0.2. Risk factors that had univariate associations ($P < 0.2$), were subsequently offered to a multivariable model through a manual backward stepwise process. Evaluating the effect of the removed variables on the coefficients of the remaining variables was used to assess confounding. A variable was deemed to be a confounder if it was not an intervening variable based on the causal diagram and the log odds of a significant variable in the model changed by at least a 20%. Two-way interactions were evaluated between biologically important variables and remained in the final models if significant ($P < 0.05$) (Dohoo et al., 2010). The model fit was assessed using Pearson and deviance χ^2 tests. Outliers were identified and evaluated using Pearson residuals and deviance residuals as well as delta-betas, delta-χ^2 and delta-deviance. If outliers were found, they were explored to determine the characteristics of the observations that made them outliers and ensure data were not erroneous.

RESULTS

Sampling

There were 11,664 unique farm codes representing 'active' producers that were sent a letter of invitation. In total, 1,373 producers began the questionnaire and agreed to participate online, however only 1,025 producers completed the full questionnaire by all means. The response proportion was therefore 9% with the completion proportion being 75%. Seventy-nine (8%), 224 (22%) and 722 (70%) participants completed the questionnaire via telephone, paper, and on-line, respectively.

Respondents

There were respondents from each of Canada's 10 provinces, with the largest portion coming from Quebec and Ontario (Figure 2.2). The respondents classified themselves as owner (87%, n=1006), manager (7%, n=83), farm worker (2%, n=23) or other (3%, n=39). Respondents designated themselves into the age categories of < 29 (14%, n=163), 30 to 49 (49%, n=564) and

greater than or equal to 50 years of age (37%, n=425). The respondents' highest level of education was high school or less for 35% (n=402), college or university for 63% (n=730) and post-graduate education for 2% (n=21). The majority of the respondents were aware of the Code of Practice (82%, n = 908). Of those who were aware, 39% (n=354) had not consulted the code in the past year, 39% (n=349) had consulted it once in the past year and 22% (n=197) had consulted more than once in the past year. The Internet was used to access dairy information by 87% (n = 911) of respondents.

The respondents had an average of 54 lactating cows at the time the survey was completed which is smaller than the average Canadian herd size of 72 lactating cows (Canadian Dairy Information Center, 2016). Most of the respondents housed their cattle in tie stall barns with the remainder being in free stall or other housing styles (Table 2.1). All male calves were sold within 2 weeks of birth by 59% (n=636) of respondents and all male calves were raised beyond two weeks of age by 9% (n=100) of respondents with the remaining respondents (32%, n=338) reporting a combination of selling and raising the male calves. A total of 63% (n=679) of respondents had at least one animal euthanized on their farm during the previous year. When euthanizing pre-weaned heifers, weaned heifers and cows, 93% (n = 619) of respondents used an acceptable method of euthanasia based on the Code of Practice (Gun shot, captive blot or veterinarian) and 7% (n=48) used an unacceptable method of euthanasia (blunt force trauma).

Euthanizing Male Calves at Birth

Five percent of respondents (n=49) answered that they had euthanized at least one male calf at birth during the previous 12 months. Among these, the proportion of male calves euthanized on the farm ranged from 1% to 100% with an average of 19% being euthanized at birth. The variables unconditionally associated with euthanizing male calves at birth were geographical region, herd size and education level.

In the final multivariable model, 3 variables were significantly associated with euthanizing at least 1 bull calf in the last year (Table 2.2). Herd size, geographic region and education level were associated with being more likely to euthanize male calves at birth. There was no significant interaction or confounding present in the model. There was a single outlier which when removed, changed the magnitude but not the direction of the coefficient for the post-graduate category in the education level variable. The outlier was retained in the final model due to the small number of observations in the post-graduate category.

Of those respondents that euthanized their male calves at birth, thirty-four percent euthanized the calf using blunt force trauma. Univariable exact logistic regression models were built to further explore reasons for euthanizing with blunt force trauma. Being located in the province of Quebec (OR=20.6 (2.5-Infinity), P <0.01) was significantly associated with using blunt force trauma at birth. Respondents were less likely to report using blunt force trauma if they reported using an acceptable method of euthanasia on cows and heifers (OR=0.01 (0.00-0.06), $P \leq 0.001$), milking 80 to 133 cows (OR=0.09 (0.00-0.80), $P \leq 0.05$), or consulting the code of practice more than once in the past year (OR=0.11 (0.00-1.04), P =0.05).

Colostrum Management

Ninety-one percent of producers responded that they always feed colostrum to male calves. The variables unconditionally associated with always feeding colostrum were age, herd size, euthanizing at birth, awareness of the code of practice and the reported importance of the veterinarian as a source of dairy health and management information.

In the final multivariable model for always feeding colostrum to male calves, 4 variables were significant (Table 2.3). Older respondents and those who had consulted the code of practice more than once in the previous 12 months were more likely to always feed colostrum to male calves. Respondents with medium to larger herds and those who reported euthanizing at least a male calf at birth were less likely to feed colostrum. There was a single outlier identified. It did not affect the magnitude and direction of the coefficients in the model and thus was not removed.

Navel Dipping

Nearly forty percent (n=418) of respondents reported that they always navel dipped male calves. The variables unconditionally associated with always navel dipping were geographical region, euthanizing bull calves at birth, awareness of the code of practice, use of the internet as a source of dairy information, the veterinarian being reported to be a very important source of information and herd size.

In the multivariable model, 3 variables were significant (Table 2.4). Navel-dipping varied by region, use of the Code of Practice and the internet for dairy information. There were 8 outliers identified that shared the same covariate pattern. When the observations responsible for this covariate pattern were removed, the magnitude and direction of the coefficients in the model did not change and thus the observations were left in the model.

Vaccination

Approximately twelve percent (n = 123) of respondents indicated that male calves were always vaccinated. Geographical region, education category, selling greater than 50% of male calves prior to two weeks of age, veterinarian being a very important source of information and herd size were offered to a multivariable model.

In the final multivariable model, region, age of sale of male calves, and reporting the veterinarian as an important source of information were associated with vaccination of bull calves (Table 2.5). One outlier was identified but did not change the magnitude or direction of the coefficients and thus was kept in the final model.

Feeding

Eighty-three percent (n = 880) of respondents reported always feeding male calves the same or more as heifer calves of the same age. The variables offered to the multivariable model were geographical region, age category, euthanizing at birth, awareness of the code of practice, use of the Internet as a source of dairy information and herd size.

In the final multivariable model, Region, practicing euthanasia at birth and herd size were associated with male calf feeding practices (Table 2.6). There were five outliers identified that were represented by a single covariate pattern. When removed from the model, the magnitude but not the direction of the euthanized at birth variable changed. However, these outliers remained in the model due to the small number of respondents that euthanized at birth.

DISCUSSION

On Canadian dairy farms there exists a large amount of variation regarding the management of male calves in early life. The majority of respondents reported that male calves always received colostrum and were always fed the same or more as heifer calves. A minority of respondents always navel-dipped male calves, vaccinated male calves and euthanized male calves at birth. To our knowledge, this is the first study to describe management practices associated with male calves on Canadian dairy farms.

A small number of respondents reported that they had euthanized at least a single male calf at birth within the previous year. A major factor that could impact the decision to euthanize is economics. There is significant variability in the price paid to Canadian producers for male calves both within and between years (Figure 2.3). With an uncertain economic climate, some producers may decide to euthanize male calves due to the inability to generate a profit once the

calf is housed and fed for several days prior to sale. In the Atlantic provinces, especially Newfoundland, a lack of accessibility to facilities that either raise male calves or market fully grown calves further compounds the economic challenges faced by dairy producers making decisions regarding male calf euthanasia. As respondents in the Atlantic provinces had much higher odds of euthanasia at birth, it is likely that this economic factor explains this practice in this region of Canada. The inability to account for this economic factor is a limitation of this study and is likely a major confounder of the regional effects.

There are a number of other reasons that could explain euthanizing calves at birth. Dystocia can inflict severe injury and pain resulting in poor vitality in the newborn calf (Murray and Leslie, 2013) and as male dairy calves experience dystocia commonly (Olson et al., 2009), it is likely that some of the respondents euthanized male calves due to their poor chance of survival. With dystocia occurring in more than 10% of calvings in North America (Mee, 2008), larger herds have a greater probability of having difficult calvings resulting in more calves with poor vitality requiring euthanasia. This may be the reason that larger herds were associated with euthanizing at least one male calf at birth. Post-graduate educated respondents reporting a greater likelihood of euthanizing bull calves at birth was surprising. As the type of degree was not specified, they could have been veterinarians or animal science graduate that recognized that calves born with poorer vitality would likely have a difficult time recovering.

A disturbing result was the reported use of blunt force trauma as a method of euthanasia for male dairy calves at birth (n=16) and female dairy animals (n=48). It is not an acceptable method of euthanasia for neonatal calves as the anatomical features make it difficult to achieve immediate destruction of brain tissue and it is also challenging to apply this method consistently (Leary et al., 2013). This method of euthanasia is also not deemed an acceptable method in the Canadian Code of Practice for the Care and Handling of Dairy Cattle. Quebec farms reported performing this method of euthanasia at birth more commonly compared to other provinces. The reasons for this practice differing by geographical region need to be evaluated to eliminate the use of this method. Larger farms tended to euthanize male calves at birth with an appropriate method of euthanasia, and thus, it is possible that they euthanize more animals and might be more confident with using other methods of euthanasia such as gunshot. As the Canadian Code of Practice for the Care and Handling of Dairy Cattle explicitly describes the proper methods of euthanasia (National Farm Animal Care Council, 2009), it is not surprising that consulting the

code more frequently was associated with a lower odds of euthanizing male calves at birth with blunt force trauma. It remains unclear whether the respondents who read the code are more welfare conscious, and thus sought out the correct method of euthanasia to ensure rapid loss of consciousness and death or were just more compliant with regulations. Regardless, these findings need to be further explored to ensure that producers are educated on alternative and appropriate humane methods of euthanasia, so that the appropriate interventions are used on all farms.

Despite overwhelming evidence surrounding the importance of colostrum management and feeding, 9% of respondents answered that they did not always feed colostrum to male calves. It could be speculated that this is an underestimation of the number that do not feed colostrum to male calves due to presence of desirability bias. Due to the crude nature of the question, it is difficult to comment on the favorability of the colostrum feeding practices to male calves, as there was no information collected on the quality, quantity, quickness and cleanliness of colostrum management. By the way of comparison to the United States, Shively et al. (2016) reported that a higher proportion of producers fed male calves colostrum, with only 4% of male calves not receiving colostrum. They also discovered many differences that existed between female and male calves in regards to colostrum management. Male calves received a smaller volume of colostrum, had delayed colostrum feeding and were left to suckle the colostrum from their dam more commonly than female calves. A study conducted in Canada also showed differential treatment of male calves (Fecteau et al., 2002), where male calves were more likely to receive colostrum with higher bacterial counts than female calves.

There were several variables associated with colostrum feeding to male calves. Being in an older age category was positively associated with always feeding colostrum. One possibility is that some of the younger respondents may not fully understand the importance of colostrum, or due to the fluctuating prices paid for male calves producers may deem them to not be a high priority as there are many other important focuses in dairy farming. The latter theory could also be used to explain why larger farms were less likely to always feed colostrum to male calves. The Canadian Code of Practice for the Care and Handling of Dairy Cattle states that calves must receive at least four liters of good quality colostrum within 12 hours of birth, with the first meal occurring as soon as possible, and no more than six hours after birth (National Farm Animal Care Council, 2009). Hence, it is not unexpected that consulting the code more frequently was associated with always feeding male calves colostrum. Both theories presented earlier could also

apply here, in that those consulting the code ensured they met the requirements or they are more welfare conscious and recognize the importance of feeding colostrum. All of these factors need to be further explored to ensure male calves always receive colostrum in order to help improve male calf health and welfare.

Although navel dipping is a relatively common practice, nearly 40% of respondents reporting that navels of all bull calves were dipped, there is a surprising paucity of data in the literature to support its use. Grover et al. (2011) demonstrated that there might be some benefit to utilizing a navel dip to control omphalitis, however, the utilization of historical controls in that study makes the interpretation of the findings difficult. Other studies have shown that the navel dipping is associated with an increase in the risk of pneumonia (Waltner-Toews et al., 1986; Windeyer et al., 2014). Both of these studies concluded that this topic requires additional research. In this current study, there were differences in navel dipping with regards to geographical region. It could be speculated that these differences are due to the availability of educational resources for producers in those regions. The Internet can be a great resource for dairy information but can also provide incorrect and misleading information. Although there is considerable anecdotal information on the Internet regarding the importance of navel dipping, it was surprising to find that those producers more likely to access the Internet were less likely to navel dip. One of the recommended practices of the code of practice is to dip calf navels in disinfectant as soon as possible after birth. This statement could have influenced producers, whom have read the code of practice, to apply navel dip to all calves.

Very few respondents vaccinated their male calves on the dairy farm. The lack of vaccination was especially true if the respondents were from Quebec and sold their male calves prior to 2 weeks of age. However, if the respondents viewed their veterinarian as an important source of information, they were more likely to vaccinate their male calves. As previously stated, there is evidence to support the use of intra-nasal vaccination of young calves and its disease sparing effect against viral pathogens in the BRD complex. A model that has been used successfully in beef production to aid in the reduction of the incidence of BRD, is for cow-calf producers to vaccinate their animals 2-3 weeks prior to entering the feedlot (Cusack et al., 2003). This is a potential model that could be used in male dairy calves to control BRD but there needs to be a premium paid to the dairy producers who implement this practice.

Although the majority of respondents reported always feeding male calves the same or more as female calves the same age, there were still a large number of respondents (n=179) that did not always feed male calves the same. This was an anticipated result as many male calves enter rearing facilities with a lack of subcutaneous fat covering their frame or even an emaciated appearance (Wilson et al., 2000). Poorer nutrition of male calves compared to female calves was also subjected to regional variation with farms in Quebec showing greater likelihood of differential feeding of male calves. Having euthanized a male calf at birth was also associated with reduced nutrition. As lower weight at arrival is one factor that is a significant predictor of mortality in veal operations (Winder et al., 2016), nutrition and age at transport need to be further explored in order to reduce the levels of mortality occurring on veal and dairy beef farms.

CONCLUSION

The major findings of this survey demonstrate that the standard of care for male calves needs to be enhanced on some Canadian dairy farms. The issues of colostrum management and early nutrition need to be further explored, to aid in reducing morbidity and mortality with the ultimate goal of improving the male calf welfare. An interesting finding was that the care of male calves differed greatly depending on the geographical region of the respondents. We hypothesize that some of the regional effects that we found may be confounded by different economic conditions and logistics of marketing male dairy calves in different parts of the country. Another theme that continued through the paper was larger farms having differences in welfare practices. They were more likely to use an appropriate method for euthanizing male calves at birth but larger farms were also associated with being less likely to always feed colostrum and not to feed male calves the same as female calves. As the trend toward larger farms continues, it is important to further explore reasons for these practices. The consultation of the Canadian Code of Practice for the Care and Handling of Dairy Cattle by respondents was consistently associated with improved care of male calves on dairy farms. As this document provides producers with valuable information impacting dairy cattle welfare, efforts need to be made to increase its impact in regions where it is not known or used.

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Table 2. 1. Proportion (%) of respondents and Canadian dairy farms (Canadian Dairy Information Center 2016; CDIC) using different housing systems

Housing Style	Proportion (no.) of respondents housing style	Proportion (no.) of farms housing style reported by CDIC in 2016
Tie-Stall	59 (674)	71 (5937)
Free-Stall	39 (442)	22 (1878)
Other	2 (28)	

Table 2. 2. Results of a multivariable logistic regression model of euthanizing at least one neonatal male dairy calf in the previous year, as reported by respondents to a survey of Canadian dairy farmers

Variable	Description	n	Odds Ratio	95% CI	*P*
	Intercept				
Geographical Region	Ontario	320	Ref.		
	Quebec	416	1.28	0.53 - 3.11	0.59
	West	119	1.35	0.46 - 3.98	0.58
	Atlantic	67	6.43	2.47 - 16.76	<0.001
Education Level	High School	298	Ref.		
	College or University	605	1.97	0.82 - 4.71	0.13
	Post Graduate	19	6.66	1.43 - 30.92	0.02
Herd Size	10 to 38	227	Ref.		
	39 to 54	226	1.94	0.52 - 7.14	0.32
	55 to 87	238	2.78	0.84 - 9.22	0.095
	88 to 888	231	5.31	1.66 - 17.00	<0.001

Table 2. 3. Results of a multivariable logistic regression model of always feeding colostrum to male dairy calves, as reported by respondents to a survey of Canadian dairy farmers

Variable	Description	No.	Odds Ratio	95% CI	*P*
	Intercept				
Age	Under 30	128	Ref.		
Category	30 to 49	443	2.07	1.13 - 3.80	0.02
	Over 49	322	2.28	1.17 - 4.44	0.02
Herd Size	10 to 38	219	Ref.		
	39 to 54	222	0.40	0.19 - 0.85	0.02
	55 to 87	225	0.55	0.25 - 1.20	0.13
	88 to 888	227	0.43	0.20 - 0.93	0.03
Aware of	No	156	Ref.		
Code of	Yes, not consulted in last 12 months	286	1.87	0.94 - 3.72	0.08
Practice	Yes, consulted once in last 12 months	288	1.15	0.61 - 2.18	0.66
	Yes, consulted more than once in last 12 months	163	2.93	1.18 - 7.27	0.02
Euthanize at	No	858	Ref.		
birth	Yes	35	0.36	0.14 - 0.93	0.03

Table 2. 4. Results of a multivariable logistic regression model of always navel-dipping male dairy calves, as reported by respondents to a survey of Canadian dairy farmers

Variable	Description	No.	Odds Ratio	95% CI	*P*
	Intercept				
Geographical	Ontario	309	Ref.		
Region	Quebec	404	1.48	1.07 - 2.05	0.01
	West	112	0.36	0.22 - 0.62	<0.001
	Atlantic	62	0.57	0.31 - 1.05	0.07
Aware of	No	156	Ref.		
Code of	Yes but not consulted in last 12 months	284	0.96	0.62 - 1.49	0.86
Practice	Yes and consulted once in last 12 months	286	1.42	0.94 - 2.14	0.10
	Yes and consulted more than once in last 12 months	161	2.21	1.34 - 3.64	<0.001
Internet for	No	76	Ref.		
Dairy Info.	Yes	811	0.52	0.32 - 0.86	0.01

Table 2. 5. Results of a multivariable logistic regression model of always vaccinating male dairy calves, as reported by respondents to a survey of Canadian dairy farmers

Variable	Description	No.	Odds Ratio	95% CI	*P*
	Intercept				
Geographical Region	Ontario	306	Ref.		
	Quebec	405	0.42	0.23 to 0.77	0.01
	West	112	1.59	0.87 to 2.91	0.12
	Atlantic	61	0.92	0.37 to 2.28	0.86
Raise or Sell	Sell <50%	99	Ref.		
	Sell >50%	785	0.11	0.07 to 0.19	<0.001
Vet Importance	Not a very important source	191	Ref.		
	Very important source	693	2.13	1.09 to 4.15	0.03

Table 2. 6. Results of a multivariable logistic regression model of always feeding male dairy calves the same or more as heifer calves the same age, as reported by respondents to a survey of Canadian dairy farmers

Variable	Description	No.	Odds Ratio	95% CI	*P*
	Intercept				
Geographical Region	Ontario	310	Ref.		
	Quebec	404	0.29	0.18 - 0.45	<0.001
	West	114	1.5	0.66 - 3.43	0.34
	Atlantic	62	0.65	0.29 - 1.50	0.32
Euthanize at birth	No	855	Ref.		
	Yes	35	0.43	0.19 - 0.96	0.04
Herd Size	10 to 38	217	Ref.		
	39 to 54	222	0.62	0.37 - 1.04	0.07
	55 to 88	228	0.55	0.32 - 0.92	0.02
	89 to 888	223	0.6	0.33 - 1.09	0.09

Figure 2. 1. Causal diagram describing the relationship of measured variables to treatment of male calves on Canadian dairy farms
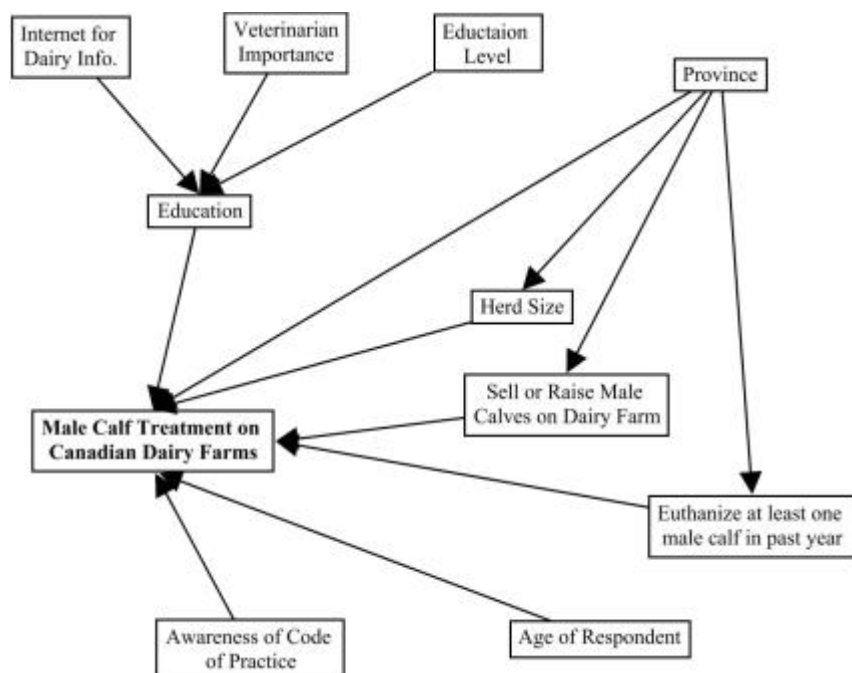
Figure 2. 2. Proportion of respondents (gray; with 95% CI) and Canadian producers (black; Canadian Dairy Information Center, 2016) by geographical regions
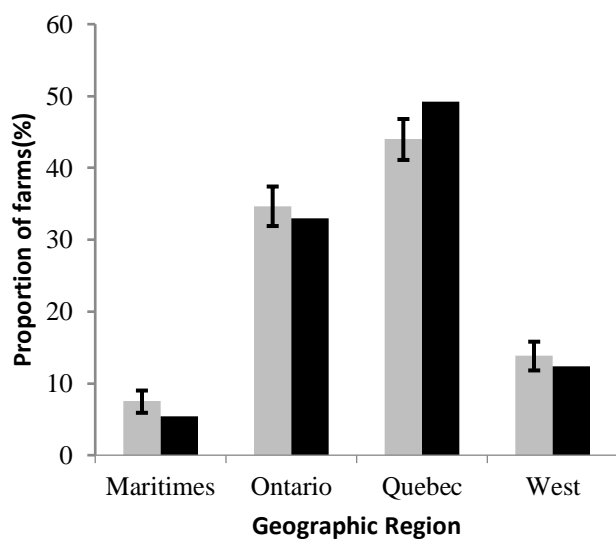
Figure 2. 3. Price paid per male dairy calf <125lbs at Quebec, Ontario and Nova Scotia auction facilities by year (Atlantic Stockyards Limited, 2016; Beef Farmers of Ontario, 2016; Les Producteurs de bovins du Quebec, 2016)
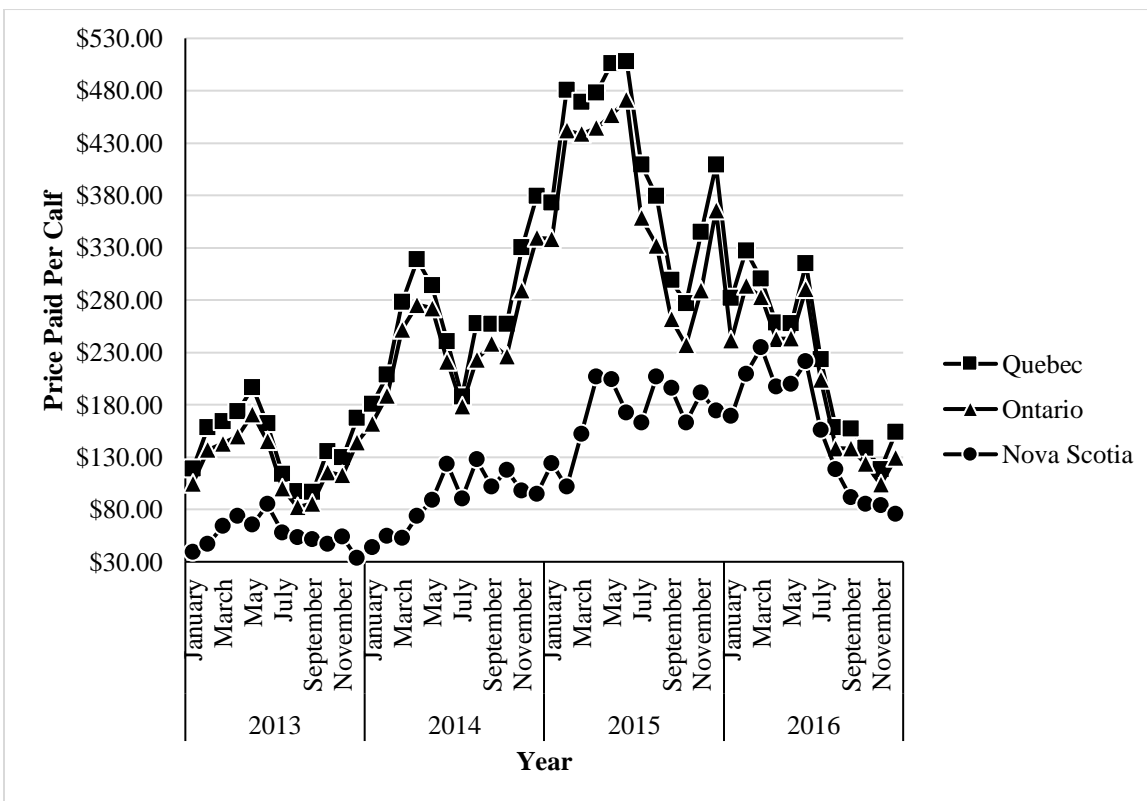
CHAPTER 3: CALF MANAGEMENT RISK FACTORS ON DAIRY FARMS ASSOCIATED WITH MORTALITY ON VEAL FARMS

As previously published

CALF MANAGEMENT RISK FACTORS ON DAIRY FARMS ASSOCIATED WITH MORTALITY ON VEAL FARMS

ABSTRACT

The objective of this cross-sectional study was to assess the association of calf management practices with high and low mortality source dairy farms as classified using mortality risk calculated at veal farms the source dairy farms supplied. From April to October 2016, 52 source dairy farms supplying male calves to two veal operations were visited once. A questionnaire was administered covering all areas of calf management, calves between 1 and 10 days of age were examined using a standardized health scoring system, and blood was taken to evaluate passive transfer of immunoglobulins. The mortality risk for calves from each dairy farm was calculated based on the number of male calves sold from the dairy farm and that died during 2016 at the veal operations. The mean mortality risk was calculated for both veal farms, and based on the facility-adjusted mortality risk, dairy farms were classified as high or low mortality source farms. Using the information gathered at the 52 source dairy farms, a logistic regression model was used to assess factors associated with being a high mortality source farm. Suppliers to veal farm 1 had a mean mortality risk of 9.6% and suppliers to veal farm 2 had a mean mortality risk of 4.2%. The lower mortality risk at veal farm 2 was partially influenced by the shorter period under observation. Of the 182 calves examined during the single visit to the source dairy farms, 41% of male calves and 29% of female calves had at least one identifiable health abnormality. The risk of failure of passive transfer on source dairy farms was low, with only 13% of calves tested having <10 mg IgG/ml of serum. The subset of calves examined at the source dairy farm were not followed prospectively to the veal farm. Using a tube feeder or pail to feed colostrum, bedding male calves on wood shavings or chopped straw at the source dairy farm, and the herd veterinarian not routinely and actively inquiring about the health and performance of calves during regular herd visits were significantly associated with the farm being classified as a high-mortality source dairy farm. Checking the calving pen at an interval of every 3 hours or more during the day was associated with a lower probability of being classified as a high-mortality source dairy farm. The results of this study suggest that there are management practices on the source farm that contribute to the risk of mortality on veal farms.

INTRODUCTION

Management of the newborn dairy calf is essential to its survival and productivity. Calving management, colostrum management, feeding, housing, and timely treatment on dairy farms are critical to the calf, including male calves. Male calves transferred to veal farms often face disease challenges resulting from transportation stress (Mormede et al., 1982), commingling with calves from multiple sources (van der Fels-Klerx et al., 2000), placement into a new housing facility and adapting to a new diet, all within the first two weeks of life. Currently, high levels of mortality (Bähler et al., 2012; Pardon et al., 2012a), morbidity (Pardon et al., 2012a), antimicrobial use (Bos et al., 2013; Pardon et al., 2012b) and antimicrobial resistance (Catry et al., 2016) are challenges faced in the veal industry. With an increased focus on improving animal welfare and an increased pressure to reduce antimicrobial use (Pardon et al., 2014), emphasis needs to be placed on prevention of disease. There are many areas that could be explored for disease prevention but as the majority of mortality occurs in the first 21 days on the veal farm (Bähler et al., 2012; Pardon et al., 2012a; Winder et al., 2016), calf management on the source dairy farm may play a role. Currently, there is a lack of peer-reviewed literature identifying management factors on dairy farms that impact calf health at veal farms despite many veal producers being empirically able to identify high mortality source dairy farms.

Because male calves are more likely to experience dystocia (Olson et al., 2009) and with approximately 10% of calvings resulting in dystocia (Mee, 2008), it is imperative to have appropriate timing and methods of intervention through regular surveillance (Mee, 2004) to reduce many of the impacts associated with dystocia.

Successful passive transfer of immunity is essential for calves in the prevention of disease (Postema and Mol, 1984; Pardon et al., 2015). To achieve adequate passive transfer of immunoglobulins, it is necessary to provide an adequate quantity of high quality colostrum quickly after birth while minimizing bacterial contamination (Godden, 2008). Despite its known importance, failure of passive transfer is estimated to affect 41 to 43% of male dairy calves (Wilson et al., 2000; Pardon et al., 2015). There have been a few studies describing some discrimination against male calves in regard to colostrum management, with male calves being more likely to be fed colostrum contaminated with bacteria (Fecteau et al., 2002), receive a smaller volume of colostrum, have delayed colostrum feeding, or be left with the dam as a mechanism to feed colostrum (Shively et al., 2016). Given the role that colostrum management

has been demonstrated to play in disease prevention (Donovan et al., 1998; Wells et al., 1996; Windeyer et al., 2014) but also in improved rate of gain and feed efficiency in female dairy calves (Faber et al., 2005; Robinson et al., 1988), reasons for this discriminatory behaviour and its impact on male calf mortality need to be described.

Several studies have shown the importance of early life nutrition. Nutritional programs providing higher volumes of milk have shown increased weight gain, and may aid in improving disease resistance (Todd et al., 2017; Khan et al., 2007; Ollivett et al. 2012). As a lower weight at arrival at a veal facility has been associated with greater risk of mortality (Brscic et al., 2012; Winder et al., 2016), nutrition might be an important factor in prevention of morbidity and mortality in male calves.

Housing style (Waltner-Toews et al., 1986), ventilation (Windeyer et al., 2014) and bedding type (Lago et al., 2006) have been shown to be associated with female dairy calf mortality. The effect of housing for male calves on the source farm on health in veal operations is largely unknown.

The objective of this cross-sectional study was to assess the association of calf management practices with high and low mortality source dairy farms as classified using mortality risk calculated at veal farms the source dairy farms supplied. A secondary objective was to identify management differences between male and female dairy calves.

MATERIALS AND METHODS

Experimental Design

A cross-sectional study was conducted from April to October 2016 to collect data on calf management practices at source dairy farms supplying calves to two veal facilities in Ontario, Canada. The two veal operations were selected due to the proximity to the University of Guelph and willingness to participate in this study. Veal farm 1 is a milk-fed veal facility that uses group and individual to group housing. This farm ships calves to slaughter for white veal after a production period of approximately 20 weeks and had a mean hot carcass weight of 280 lbs for all calves slaughtered in 2016. Veal farm 2 is a grain-fed veal facility that uses individual to group housing for all calves reared. After 11 weeks at veal farm 2, the calves are shipped to a group housing facility and are slaughtered after an additional 20 weeks for red veal. The mean hot carcass weight was 380 lbs for all calves slaughtered in 2016 from veal farm 2. Both veal

facilities maintained the housing facilities at a minimum of 15°C in the initial portion of the growing period and housed the calves on a slatted floor.

The source dairy farms were visited once and each visit involved the completion of a questionnaire, examination of calves between 1 and 10 days of age using a standardized health scoring system and collection of blood to evaluate passive transfer. The questionnaire (Appendix 1) was comprised of 75 questions addressing herd demographics, calving management, newborn calf care, colostrum management, calf housing and feeding, dry cow management and veterinary assistance. The questionnaire was developed through a literature review of the main factors impacting calf health and based on questions developed by Vasseur et al. (2010). All questions were asked verbally to each participating dairy producer and their responses were entered into an online platform (Qualtrics™(https://www.qualtrics.com/)) by one of the investigators during the visit. The questionnaire specifically inquired about differences in male and female calf management. The standardized health screening was completed using the Calf Health Scorer application developed by the University of Wisconsin. This application uses validated methods for fecal scoring (McGuirk, 2008), respiratory screening (McGuirk and Peek, 2014) and navel inflammation (adapted from Fecteau et al., 1997). Following the health examination, approximately 10 ml of whole blood was collected from the jugular vein into a sterile blood collection tube without an anticoagulant (BD Vacutainer; Becton, Dickson and Co., Franklin Lakes, NJ, USA). Blood samples were allowed to clot and then centrifuged at $1,500 \times g$ for 15 minutes at approximately 20°C. Serum was separated and stored at -20°C until submission Saskatoon Colostrum Company (Saskatoon, SK, Canada) for analysis of serum IgG by radial immunodiffusion as described by Chelack et al. (1993). Calves were classified as having failure of passive transfer (FPT) if their serum IgG was < 10 g/L (Godden, 2008). The subset of calves examined at the source dairy farm were not followed at the veal farms.

Human ethics (REB#16MR023) and animal use (AUP#3453) approvals were received from the University of Guelph prior to beginning the on-farm portion of the study.

Dairy producers who sold more than 5 male calves in 2015 to veal farm 1 or veal farm 2 were eligible for participation in the study. The participants were contacted by telephone and provided with information regarding the purpose and terms of participation in the project. After receiving consent from the dairy producer, an on-farm visit was conducted. To ensure that confidentiality was maintained, a unique anonymous code was assigned to each producer

following the on-farm visit. For those that declined to participant, no information was made available to compare the responders to the non-responders.

Statistical Analysis

All statistical analyses were conducted using Stata 14 (StataCorp, 2015). Data were imported from Microsoft Excel into Stata 14 and checked for completeness. A causal diagram (Figure 3.1) was created to evaluate the relationships between the potential explanatory variables and the outcomes of interest. Descriptive statistics were generated for all explanatory variables in the dataset. For questions that specifically inquired about differences between male and female calf management practices, a two-sample z-test was performed to compare proportions.

A facility-adjusted mortality risk was calculated for each veal farm based on the total number of calves that were received and died in 2016 during the rearing period of 20 weeks or 11 weeks for veal farm 1 and 2, respectively. A mortality risk was also calculated for each source dairy farm based on the number of male calves sold and the number that died in 2016 at the veal operations. Based on the 2016 facility-adjusted mortality risk, source dairy farms were classified as high (HMSF) or low (LMSF) mortality source farms. A source dairy farm was classified as a HMSF if the farm mortality risk was greater than the veal facility-adjusted mortality risk that the source dairy farm shipped male calves to. A source dairy farm was a LMSF if the farm mortality risk was lower than the veal facility-adjusted mortality risk.

One explanatory logistic regression model was created to identify characteristics of the HMSF versus LMSF and one explanatory Poisson regression model was built to explore herd-level factors associated with rate of FPT at each source dairy farm visited using the blood collected from the calves during the single farm visit. The logistic model assumption of linearity of continuous variables was assessed by plotting the logarithmic odds of the outcome against the variable, whereas, the assumption of linearity in the Poisson model was tested by plotting the natural logarithm of the outcome against the continuous variable. If a variable failed to meet the linearity assumption, the variable was categorized. In the logistic model, calving observation during the day, cow to calf contact after calving and time to collect colostrum after calving were dichotomized at the median response. Herd size was categorized into quartiles in both the Poisson and logistic models. Collinearity among the explanatory variables was tested using Spearman rank coefficients. If the correlation coefficient between 2 variables was ≥ 0.7, only one variable was retained based on fewest missing values, reliability of measurement and biological

plausibility. Univariable logistic regression models were constructed to screen for variables that were unconditionally associated with the outcome using a *P*-value of 0.2. Risk factors that had univariate associations ($P < 0.2$) were subsequently offered to a multivariable model through a manual forward stepwise process. Confounding was assessed by evaluating the effect of removing variables on the coefficients of the remaining variables. A variable was deemed to be a confounder if it was not an intervening variable based on the causal diagram and the log odds of a significant variable in the model changed by at least 20% when the potential confounder was added to the model. Two-way interactions were evaluated between variables suspected to interact based on evidence from the literature and remained in the final models if significant (P-value < 0.05) (Dohoo et al., 2010a). The logistic and Poisson model fit was assessed using Pearson and deviance χ^2 tests. These tests were used in the logistic model as solely binomial data (i.e. leading to the number of covariate patterns to be much lower than the number of data points in the dataset) was included in the model (Dohoo et al., 2010b). Outliers were identified and evaluated using Pearson residuals and deviance residuals in both models. Additionally, in the logistic model, delta-betas, delta-χ^2 and delta-deviance were explored, whereas, in the Poisson model Anscombe residuals and Cook's distance were evaluated. If outliers were found, they were explored to determine the characteristics of the observations that made them outliers and ensure that data were not erroneous.

RESULTS

Herd Demographics

A total of 73 and 63 source dairy farms selling calves to veal farm 1 and 2, respectively, were contacted to participate in the study. Of those contacted, 23 (32%) source dairy farms supplying veal farm 1 and 29 (46%) source dairy farms supplying veal farm 2 agreed to participate in the study. The participating source dairy farms had an average of 135 lactating cows, which is larger than the average Canadian herd size of 72 lactating cows (Canadian Dairy Information Center, 2016). The housing style used by participants was also different from Canadian dairy farms (Canadian Dairy Information Center, 2016) as the majority milked the cows in free-stall barns (73%) and the remainder used tie-stall (25%) or bedded packs (2%) for housing.

Calving Management

Most participants (69%) used group calving pens with an average of 92% of calvings occurring in the designated calving area. Only 12% of producers washed or disinfected the calving area between calvings and 38% used the calving area for sick or lame cows. The producers checked the calving area on average every 3 h and 6 h during the day and night, respectively. Twenty nine percent of producers used calving monitoring equipment such as video cameras. Overall, 14% of producers assisted with all calvings, with 19% assisting with all calvings if calving area observations were made every ≤ 2 h during the day or 8% if the calving area was observed every ≥ 3 h during the day. The majority, however, assisted with a calving only after checking and finding a problem (64%), or when the cow was not progressing as expected (17%). Producers assisted with an average of 37% of primiparous calvings and 25% of multiparous calvings.

Newborn Calf Care

Calf care provided for newborn calves is described in Table 3.1 and did not differ between male and female calves.

Colostrum Management

Colostrum was typically fed within the first 4 hours of life and an average of 3 liters was fed to each calf. Very few respondents evaluated the colostrum quality prior to feeding and tested serum total proteins to evaluate the success of the colostrum management program (Table 3.1).

Housing and Feeding

Male calves were more likely to be housed individually ($P = 0.03$), with 71% of source dairy farms housing males individually and 54% of source dairy farms housing females individually. Bedding was added to the housing system at least twice a week and cleaned out more than every week on the majority of farms. The most commonly used bedding was long straw. Only 19% of farms kept male calves on the source dairy farm for > 7 days of age.

Most producers offered at least 6 liters or greater of milk or milk replacer to calves in the first week of life. Eighty-one percent of source dairy farms reported using solely a nipple bottle to deliver milk to male calves whereas 33% solely used a nipple bottle for female calves ($P <$ 0.001). Source dairy farms were also less likely to use a robotic feeder to deliver milk to male calves ($P < 0.001$), with only 8% using the feeder for male calves and 31% using the feeder for

female calves. Most of the producers cleaned the feeding equipment daily and did not feed waste milk to calves (Table 3.1).

Veterinary Assistance

Most producers had a veterinarian on their farm at least every 2 weeks for routine herd health visits. Farmers reported that most of the herd veterinarians did not routinely and actively inquire about the health and performance of dairy calves during regular herd visits (Table 3.1).

Veal Farm Information

Veal farm 1 received a total of 8028 calves in 2016 and 774 (9.6%) died during the 20 wk production period. A total of 23 source dairy farms for veal farm 1 were visited, with 11 source dairy farms being classified as HMSF. Veal farm 2 received a total of 1220 calves in 2016 of which 51 (4.2%) died during the 11 wk production period. A total of 29 source dairy farms for veal farm 2 were visited, with 8 being classified as HMSF. The source dairy farms shipped an average of 31 calves in 2016 (maximum of 94 and minimum of 5 calves).

Individual Calf Examination and Passive Transfer

A total of 182 calves, aged 1 to 10 days, were examined during the source dairy farm visits. Failure of passive transfer was found in 15% (n = 9) of male calves and 13% (n = 13) of female calves. The variables unconditionally associated with the herd FPT rate were colostrum source fed, having a sufficient quantity of colostrum reserves and always cleaning the colostrum feeding equipment prior to use. In the final model, colostrum source was the only remaining variable. If colostrum replacer was used a principal colostrum source for calves, those herds had a higher incidence of FPT (IRR: 2.98; 95% Confidence Interval: 1.12 to 7.93; $P = 0.03$) when compared to using fresh colostrum as a principal colostrum source for calves.

An abnormal navel score, defined ≥ 2, was found in 33% (n = 21) and 7% (n = 7) of male and female calves respectively. Overall, 41% (n = 26) of male calves and 29% of female calves had at least one identifiable health abnormality.

High Mortality Dairy Farms

The variables unconditionally associated with being a HMSF are presented in Table 3.2. In the final multivariable model, five variables remained ($P < 0.05$) (Table 3.3). Using a tube feeder or pail to feed colostrum, having a herd veterinarian who inquired less frequently about the health and performance of calves, and using wood shavings or chopped straw as bedding for male calves were all associated with a higher odds of the dairy farm being classified as a HMSF.

Checking the calving pen at an interval of every 3 hours or greater was associated with lower odds of being classified as a HMSF. The time to collect colostrum following calving was retained in the model as it changed the log-odds of the colostrum feeding method variable by greater than 20%. There were 2 outliers identified that shared the same covariate pattern, however, they were retained in the final model as the magnitude and the direction of the coefficients did not change when they were removed.

DISCUSSION

This study identified that certain management practices on source dairy farms are associated with mortality risk on veal farms. The major limitation of this study was the small number of source dairy farms visited which resulted in large odds ratios and confidence intervals. The sample size was limited due to the resources available and low response rate of the source dairy farms. The inability to identify when changes in management practices occurred and determine if they were altered as a result of previous health challenges is another limitation to the study. Some of the identified risk factors could also be proxy measures for other unmeasured factors including the genetics impact of the source dairy farms breeding strategies. Despite these limitations, this study is the first to trace male calves back to source dairy farms and identifies bedding type, frequency of monitoring the calving area, colostrum feeding method and the herd veterinarian interest in the health and performance of calves as factors associated with high mortality source dairy farms.

The mortality risk calculated for veal farm 1 was substantially higher than the risk at veal farm 2. However, veal farm 2 had a shorter production period which may have impacted the different mortality risks. Mortality risk at veal farm 1 was also higher than previously reported in Canadian studies (Sargeant et al., 1994; Winder et al., 2016) but also international studies (Bahler et al., 2012; Pardon et al., 2012a; Wilson et al., 2000). As public concern surrounding animal welfare on the rise (Spooner et al., 2014; Vanhonacker et al., 2008), this level of mortality highlights the need to explore factors to improve animal health and welfare.

Monitoring the calving area less frequently during the day was associated with lower odds of being classified as a HMSF. It could be speculated that farms with more intensive monitoring of the calving area had higher levels of obstetrical assistance, which could be related to periparturient management decisions such as sire selection, excess nutrition leading to over conditioning and for heifers weight or size at breeding (Mee, 2008). Calves that are assisted

during birth are less vigorous, have longer time to sternal recumbency and had significantly higher levels of FPT (Barrier et al., 2012; Barrier et al., 2013). As these factors have been associated with mortality (Pardon et al., 2015; Schuijt and Taverne, 1994), calving management may play a role in the prevention of mortality at veal operations.

Feeding colostrum other than by nipple bottle was associated with being a HMSF. When using an esophageal tube feeder, the esophageal groove is not triggered leading to the deposition of colostrum into the forestomachs (Godden, 2008; Lateur-Rowet and Breukink, 1983). As it takes additional time for the colostrum to transfer from the forestomachs into the small intestine, it may reduce the efficiency of colostrum absorption due to the closure of the gut (Bush and Staley, 1980). Although this challenge can be overcome with additional volume and appropriate timing (Elizondo-Salazar et al., 2011; Godden et al., 2009), the low volume of colostrum being fed by most producers could impact the level of FPT increasing the level of mortality on the veal farm (Pardon et al., 2015).

The use of a commercial colostrum replacer by the source dairy farm was associated with a higher level of FPT in the calves tested at the single farm visit. This finding is similar to other studies which demonstrated that calves fed colostrum replacer had lower levels of IgG when compared to fresh maternal colostrum (Smith and Foster, 2007; Swan et al., 2007). Despite this finding, caution should be applied in the interpretation of this model due to the low number of calves evaluated, the reliance on survey data to indicate principal colostrum source for the herd and that this was not a primary objective of this study.

The type of bedding material is important in keeping calves clean, dry, and comfortable. The use of solely long straw bedding for male calves was associated with the dairy farm having lower odds of being classified as a HMSF. Long straw allows calves to adapt to cold or drafty conditions as deep nesting may preserve nutritional stores and immune function leading to a reduction in the prevalence of respiratory disease (Lago et al., 2006). Our study took place during summer, but we expect that most farms used the same type of bedding for calves all year. Panivivat et al. (2004) also demonstrated that calves housed on long straw had the lowest number of scour days and lower coliform counts in the bedding when compared to other bedding types. As respiratory disease and diarrhea are the main causes of mortality in veal calves (Bähler et al., 2012; Pardon et al., 2012a), the use of long straw bedding for male calves on dairy farms may help to reduce some of the mortality created by these diseases.

Many studies have demonstrated that producers view veterinarians as a reliable and credible source of information (Gunn et al., 2008; Jansen and Lam, 2012; Ritter et al., 2015). Veterinarians also can initiate discussions and play a key role in implementing changes in management practices to improve disease control (Frank and Kanene, 1993; Lam et al., 2011; Jansen and Lam, 2012). A surprising finding from this study was the low number of producers who reported that their veterinarian routinely and actively inquired about the health and performance of their calves. This variable was associated with a farm being a HMSF. Veterinarians need to engage in discussions with clients regarding calf health management to identify problems early and put in place corrective management practices as well as aid in therapeutic decision-making.

There were very few differences reported between the management of male and female calves. This differs from some other studies where male calves received differing treatment when compared to female calves (Fecteau et al., 2002; Renaud et al., 2017; Shively et al., 2016). There may have been some level of information bias present, where the respondents, knowing the purpose of the study, may have selectively suppressed differences in male and female calf management. Also, non-response bias, a form of selection bias, could have also been related to the lack of differences found. However, this likely did not occur on all farms and highlights that most the respondents visited display no differences when managing male and female calves with the exception of the type of housing and method used to provide milk to the calves.

There were many calves that had health abnormalities. Many male calves had an abnormal navel score whereas the prevalence was lower in females, similar to what was reported by Virtala et al. (1996). Navel infections may cause septicemia leading to lower growth and increased mortality (Donovan et al., 1998; Virtala et al., 1996) and thus increased navel scores may play a role in mortality on veal farms.

CONCLUSION

Based on the results of this study, there are management practices occurring on dairy farms that may impact male calf health on veal farms. The use of an esophageal tube feeder or pail to feed colostrum, using wood shavings or chopped straw as male calf bedding, infrequent inquiry by the herd veterinarian about the health and performance of calves, and observing the calving area more frequently were all associated with a greater odds of the dairy farm being classified as high mortality.

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Table 3. 1. Proportion (%) of source dairy farms (n = 52) performing calf care management practices for all calves

Section	Management practice		All calves
Newborn Calf Care	Navel dip		49
	Assessing calf vigor		71
	Stimulate weak calves		92
	Suspend calves by rear legs		35
	Place calf in sternal recumbency		71
	Moved away from calving pen immediately following birth		66
Colostrum Management	Colostrum Source	Fresh	77
		Stored	13
		Replacer	11
	Timing of 1st Feeding	≤ 2 hours	39
		> 2 to ≤ 4 hours	53
		> 4 hours	9
	Quantity at 1st Feeding (L)	≤ 2	22
		> 2 to < 3	4
		≥ 3 to < 4	57
		≥ 4	18
	Provided 2nd colostrum feeding in first 12 hours		89
	Provided transition milk		52
	Method for colostrum delivery	Nipple bottle	77
		Tube feeder	13
		Pail	4
		Left with dam	2
		Combination of nipple bottle and tube feeder	5
	Disinfected feeding method prior to use		77
	Time until collection of colostrum	≤ 2 hours	33
		> 2 to ≤ 4 hours	31
		> 4 to ≤ 6 hours	24
		> 6 hours	12
	Assessed colostrum quality		18
	Evaluated total proteins for passive transfer		14

Housing and Feeding	Bedding is added twice a week or more		57
	Bedding is completely cleaned out at least weekly		16
	Housing washed with disinfectant after clean out		34
	Bedding Type	Long straw	49
		Chopped straw	12
		Wood shavings	15
		Combination	25
	Volume of milk or milk replacer provided in first week of life	$\leq$ 4L	20
		> 4 to < 6L	5
		$\geq$ 6L	76
	Method of delivery cleaned daily		82
	Used waste milk to feed calves		21
Veterinary Assistance	If unable to deliver a calf call vet		92
	Have a written protocol for disease treatment in calves		65
	Vet presence on farm	Every week	2
		Every 2 weeks	50
		Every month	37
		More than every month	8
		Never	4
	How often does the vet inquire about the health and performance of calves at routine herd visit?	Always	33
		Sometimes	21
		Seldom	21
		Never	13

Table 3. 2. Results of univariable logistic regression models evaluating risk factors for being a high-mortality source dairy farm from a single interview of 52 source dairy farms providing male calves to 2 veal farms

Variable	Description	n	Odds Ratio	95% Confidence Interval	P
Herd size	35 to 79	12	Referent		
	80 to 110	13	19.3	1.8 – 209.6	0.02
	111 to 150	14	6.9	0.7 – 70.8	0.58
	151 to 520	13	9.4	0.9 – 95.9	0.06
Calving observation during day time	$\leq$ 2 hours	25	Referent		
	$\geq$ 3 hours	24	0.3	0.8 - 4.7	0.06
Calving assistance	Routinely assist on all	7	Referent		
	After checking and finding a problem	31	0.3	0.5 - 7.1	0.13
	Not progressing	9	0.05	0.8 - 9.2	0.03
	Other	2	0.4	0.01 – 10.1	0.57
Cow-calf contact	$\leq$ 2 hours	25	Referent		
	$\geq$ 3 hours	27	0.2	0.06 – 0.73	0.01
Time to collect colostrum	$\leq$ 2 hours	17	Referent		
	$\geq$ 3 hours	34	2.6	0.7 – 9.9	0.15
Male colostrum feeding method	Nipple Bottle	39	Referent		
	Tube Feeder or Pail	13	2.9	0.8 – 11.1	0.12
Always clean colostrum feeding method	No	12	Referent		
	Yes	40	3.6	0.7 – 19.2	0.13
Length of time male calves on dairy farm	0 to 7 days	42	Referent		
	> 7 days	10	0.2	0.02 – 1.6	0.13
Source of male calf milk	Commercial Milk or Milk Replacer	40	Referent		
	Waste Milk	12	3.8	0.9 – 15.5	0.06
Frequency that veterinarian asks about health of calves	Always	17	Referent		
	Sometimes	11	11.7	1.1 – 122.4	0.04
	Seldom	11	32.6	2.9 – 374.1	<0.01
	Never	13	12.0	1.2 – 120.1	0.03
Treatment protocols for calves	No	34	Referent		
	Yes	18	0.13	0.03 – 0.69	0.02
Bedding used for male calves while on source dairy farm	Long straw	25	Referent		
	Wood shavings or chopped straw	27	5.6	1.6 – 19.9	<0.01

Table 3. 3. Results of final multivariable logistic regression model evaluating risk factors for being a high-mortality source dairy farm from a single interview of 52 source dairy farms providing male calves to 2 veal farms

Variable	Description	n	Odds Ratio	95% CI	*P*
Calving observation during day time	≤2 hours	25	Ref.		
	≥3 hours	24	0.02	0.0 – 0.7	0.03
Time to collect colostrum after calving	≤2 hours	17	Ref.		
	≥3 hours	34	7.3	0.5 – 107.3	0.15
Colostrum feeding method for male calves	Nipple Bottle	39	Ref.		
	Tube Feeder, Pail or Left with Dam	13	57.4	1.5 – 2190	0.03
Frequency that veterinarian asks about health of calves at routine herd visit	Always	17	Ref.		
	Sometimes	11	222.4	3.2 – 1522	0.01
	Seldom	11	88.3	3.1 – 2495	<0.01
	Never	13	416.8	6.2 – 2770	<0.01
Bedding used for male calves while on source dairy farm	Long straw	25	Ref.		
	Wood shavings or chopped straw	27	66.5	2.7 – 1604	0.01

Figure 3. 1. Causal diagram describing the relationship of measured variables to high mortality source dairy farms
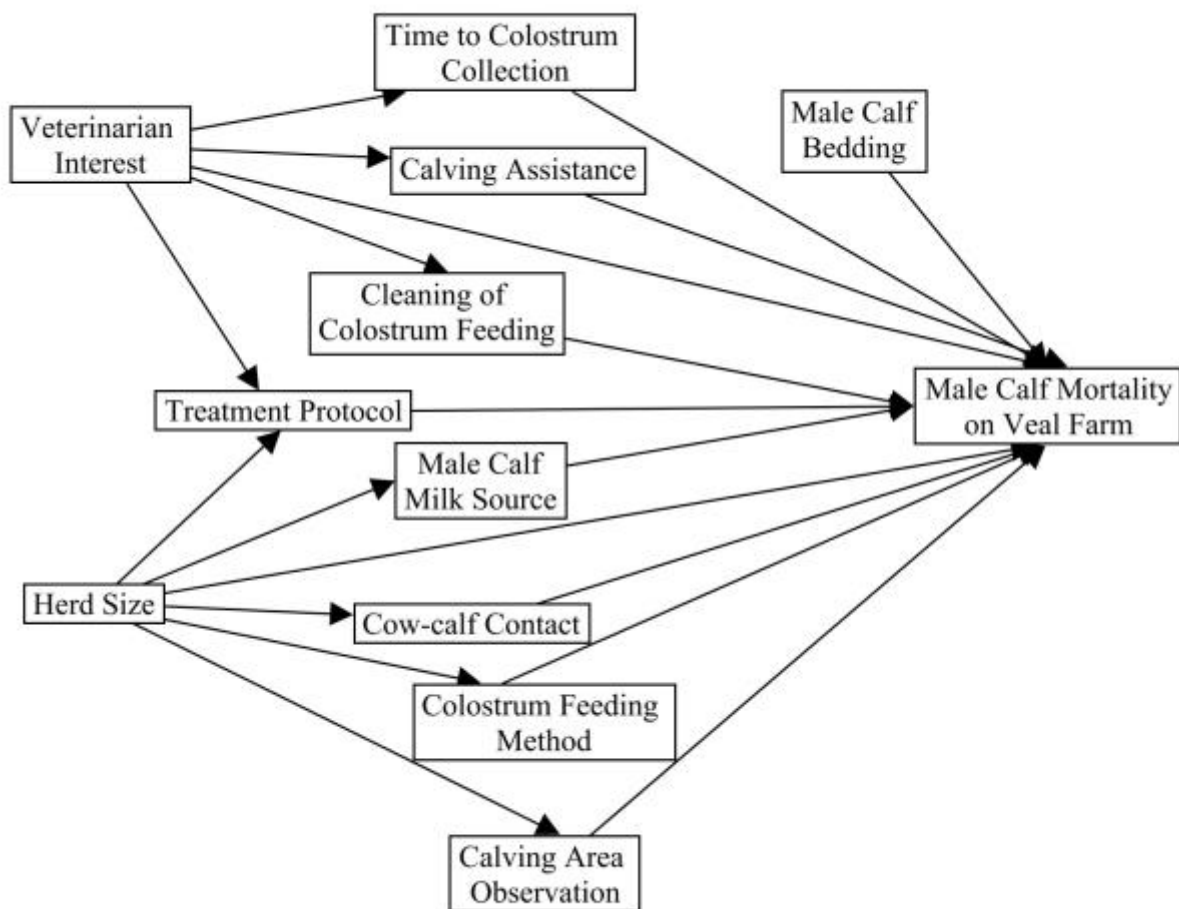
CHAPTER 4: RISK FACTORS ASSOCIATED WITH MORTALITY AT A MILK-FED VEAL FACILITY: A PROSPECTIVE COHORT STUDY

RISK FACTORS ASSOCIATED WITH MORTALITY AT A MILK-FED VEAL FACILITY: A PROSPECTIVE COHORT STUDY

ABSTRACT

The veal industry experiences calf losses during the growing period which represent a challenge to animal welfare and profitability. Health status at arrival may be an important predictor of calf mortality. The objectives of this prospective cohort study were to describe the health status of calves arriving at a veal farm and determine the risk factors associated with early and late mortality. Using a standardized health scoring system, calves were evaluated immediately at arrival to a commercial milk-fed veal facility in Ontario. Weight at arrival and the supplier of the calf were recorded. The calves were followed until death or the end of their production cycle. Two Cox proportional hazard models were built to explore factors associated with early (≤ 21 d following arrival) and late mortality (> 21 d following arrival). A total of 4,825 calves were evaluated from November 2015 to September 2016. The overall mortality risk was 7%, with 42% of the deaths occurring in the first 21 days after arrival. An abnormal navel, dehydration, housing location within the farm, arriving in the summer and the presence of a sunken flank were associated with increased hazard of early mortality. Drover-derived calves and calves with a greater body weight at arrival had lower hazard of early mortality. Housing location within the farm, being derived from auction facilities and an abnormal navel, were associated with higher hazard of late mortality. These results demonstrate that risk factors for mortality can be identified at arrival which represents a potential opportunity to selectively intervene on these calves to reduce mortality. However, methods of preventing the development of these conditions prior to arrival needs to be explored and encouraged to improve the welfare of the calves entering the veal industry.

INTRODUCTION

Mortality in dairy calves, whether female or male, represents a significant welfare issue (Ortiz-Pelaez et al., 2008) and a major source of economic loss to livestock industries. High levels of antimicrobial use (Bos et al., 2013) and resistance (Catry et al., 2016) are also among the challenges faced with the rearing of dairy calves. With public concern about animal welfare on the rise (Spooner et al., 2014; Vanhonacker et al., 2008), there is a need to improve animal health to reduce the levels of morbidity and mortality.

There is a lack of published information on male calf mortality in North America; however, estimates from the veal and dairy beef industries suggest mortality is high. Winder et al. (2016), reported a mortality risk of 8% over the entire production period at a single milk-fed facility in Ontario. Pardon et al. (2012a) reported a mortality risk of 5% on 15 Belgium milk-fed veal farms, whereas Bahler et al. (2012) reported a mortality risk of 4% in calves housed on 15 high animal welfare standard veal farms in Switzerland. As the majority of mortality occurs within the first 3 weeks following arrival at veal calf raising facilities (Bahler et al., 2012; Pardon et al., 2012a; Winder et al., 2016), initial management at the veal facility, but also management at the dairy farm of origin, may be critical in the health and welfare of calves.

Management of newborn calves on dairy farms impacts their survival and productivity, with calving management (Wells et al., 1996), colostrum management (Postema and Mol, 1984; Pardon et al., 2015), early life nutrition (Ollivet et al., 2012; Todd et al., 2017), and housing (Lago et al., 2006; Waltner-Toews et al., 1986; Windeyer et al., 2014) all playing critical roles in disease risk. Commingling, crowding, and transportation (Mormede et al., 1982; van der Fels-Klerx et al., 2000) are additional challenges faced by calves prior to arrival at veal facilities.

Similarly, management practices on veal operations have also been identified as risk factors impacting calf health. Purchasing practices, type of breed reared, housing, ventilation, herd size and nutrition (Brscic et al., 2012; Lava et al., 2016; Todd et al., 2017) have been associated with elevated mortality, morbidity and antimicrobial use at veal operations. Hence, management of calves on the veal operation plays an equally critical role in their health and welfare.

A management practice that is commonly used at arrival to veal facilities is to provide group oral antibiotics (Pardon et al., 2012b) likely due to the large number of calves that enter the veal industry with health challenges (Wilson et al., 2000). However, it is unclear if health abnormalities identified at arrival impact mortality as the sole studies (Bahler et al., 2012); Wilson et al., 2000) evaluating individual calves at arrival yielded few associations between reduced general condition at arrival and an increased risk of morbidity or mortality. If the identification of calves at high-risk for mortality could be accomplished, it may provide an opportunity to intervene selectively with antibiotics or supportive therapy while reducing overall antibiotic use (Pardon et al., 2015).

The objectives of this study were to describe the health status of calves at arrival to a veal facility and to associate characteristics of the arriving calf with early and late mortality.

MATERIALS AND METHODS

This prospective cohort study was conducted in cooperation with a milk-fed veal producer and in accordance with the University of Guelph Animal Care Committee requirements (Animal Use Protocol: #3453). The producer had 5 barns in different geographical locations within the southwestern region of the province of Ontario, Canada. In barns 1, 2 and 4 the calves were fed manually, whereas in barns 3 and 5 automatic calf feeders were used. Calves were housed individually in barns 1 and 4 and in groups of 60 calves in barns 3 and 5. Calves in barn 2 were housed in individual pens in early life, transitioning to groups of 8 calves 5 to 6 weeks following arrival.

Data Collection

When calves arrived at the barns, they were immediately evaluated with a standardized health scoring system and weighed using a digital weigh-scale (Cardinal Scale Manufacturing Co.; Webb City, MO). The supplier of the calf and receiving date were recorded. In total, there were 233 different recorded suppliers. These suppliers were divided into 7 categories (5 drovers, local, and auction). 'Local' refers to dairy farmers who delivered their calves directly to the veal facility. The term 'drover' was used for calves that were transported directly from multiple dairy farms to the veal facility by a third party and 'auction' was used to classify calves purchased by the veal farm from auction markets. Season was categorized as winter (December to February), spring (March to May), summer (June to August) and fall (September to November). Calves were identified at arrival based on their Canadian Cattle Identification Agency (CCIA) eartag. Trax-IT© software (Merit-Trax Technologies; Mount Royal, Quebec, Canada) was used to record all mortalities occurring during the production period.

Standard Health Scoring System

An iPad (Apple Inc.; Cupertino, CA) with the Calf Health Scorer app (University of Wisconsin-Madison, Madison, WI) and Qualtrics™ (http://www.qualtrics.com/) was used to record the health scoring. The calf health scorer app provided images and descriptions to evaluate the respiratory system (nose, eye, ear, cough (McGuirk and Peek, 2014), fecal consistency (McGuirk, 2008), navel inflammation (adapted from Fecteau et al., 1997), joint swelling and rectal temperature. A second recording form developed in Qualtrics™ was used to

evaluate and record dehydration (adapted from Wilson et al., 2000), body condition score (BCS) (Wilson et al., 2000) (Table 4.1) and sunken flank. Sunken flank (Bähler et al., 2012) was scored based on the appearance and palpation of the abdomen. A flank was not considered sunken if the calf had a convex appearance to the lower portion of the paralumbar fossa and fluid could be balloted. The health scores were not provided to the barn staff to ensure that the screening of the calves was not influencing treatment decisions.

All calves were examined by one of three observers. Observer 1, a veterinary practitioner, provided training to observers 2 and 3, who were veterinary students. Using scores gathered from all calves arriving at the facility on June 17th, 2016, the inter- and intra-observer agreement were calculated for Observer 1 and 2 using percent agreement (McHugh, 2012) and Weighted *K*appa (Cohen, 1968). A Fleiss-Cohen weight type was applied when calculating the Weighted *K*appa (Fleiss and Cohen, 1973). Observer 3 relocated to pursue another position and was not able to be assessed for observer agreement.

Sample Size Calculation

A proportion estimation sample size calculation was used to determine the required number of calves. Based on previous work by Winder et al. (2016) and a review of available records, it was estimated that calves identified at arrival with a health abnormality would have a mortality risk of 10% whereas those without an abnormality would have a mortality risk of 7.5%. Using 95% confidence interval and 80% power, a sample size of 4,010 calves was required.

Statistical Analysis

All statistical analyses were completed using Stata 14 (StataCorp LP, College Station, TX). Data were imported from Microsoft Excel (Microsoft®; Redmond, WA) into Stata 14 and checked for completeness. Calves with missing data were deleted from analysis (complete-case analysis; Pigott, 2001). A causal diagram (Figure 4.1) was created to illustrate the hypothesized relationship between the dependent and independent variables, and was used to guide the analyses. Descriptive statistics were generated for all explanatory variables in the dataset.

There were two explanatory Cox proportional hazard regression models created, one for each of the outcomes early mortality and late mortality. Early mortality was defined as mortality occurring $\leq$ 21 days following arrival and calves, if they survived, were censored at 21 days following arrival. Late mortality was defined as mortality occurring > 21 days after arrival where calves that survived in the first 21 days entered at day 22 following arrival and were censored, if

they survived, at the recorded date of transportation to slaughter. If the transportation to slaughter date was missing, the date was estimated from the cohort of calves that had the same arrival date. The time series variable for both models was the number of days until mortality. The cut point of mortality at 21 days was selected based on previous work which demonstrated that the majority of mortality occurred in the first 3 weeks after arrival at a veal calf facility (Bahler et al., 2012; Pardon et al., 2012a; Winder et al., 2016). Further rationale for the selection of this threshold is the prevalence of morbidity also peaks in the first 3 weeks following arrival and different risk factors were associated with morbidity in the first 3 weeks following arrival compared with the remainder of the growing period (Brscic et al., 2012; Pardon et al., 2012a). To ensure this dataset had a similar mortality pattern, a Kaplan-Meier survival estimate was created (Figure 4.2).

For each Cox proportional hazard model, the functional form of the continuous explanatory variables was assessed by computing the Martingale Residuals from a model without the continuous predictor of interest and plotting the residuals against the predictor. If the relationship was discovered to not be linear, the variable was categorized into 2 categories. Rectal temperature in both models and body weight at arrival were categorized based on cut points generated by the Youden Index (Youden, 1950). The Youden Index is a summary measure of the receiver operator curve (ROC), measuring the accuracy of a diagnostic marker and generating an optimal cut-point to maximize both sensitivity and specificity (Fluss et al., 2005). Collinearity among the explanatory variables was tested using Spearman rank coefficients. If the correlation coefficient between 2 variables was ≥ 0.6, only one variable was retained, based on the fewest missing values, reliability of the measurement and biological plausibility (Dohoo et al., 2010a). Univariable Cox proportional hazard models were constructed to screen for variables that were unconditionally associated with the outcome using a P-value of 0.2. Risk factors that were associated with the outcome were subsequently offered to a multivariable model through a manual backward stepwise removal process. Variables were retained in the multivariable models if they were significant at a $P < 0.05$. Confounding was assessed by evaluating the effect of removal of a variable on the coefficients of the remaining variables. A variable was deemed to be a confounder if it was not an intervening variable based on the causal diagram and the coefficients of a significant variable in the model changed by at least 20% when the variable was removed from the model. Two-way interactions were evaluated between variables suspected to interact based on evidence from the literature and remained in the final models if significant (P-

value < 0.05) (Dohoo et al., 2010a). The assumption of proportionality was assessed using the test of proportional assumptions (Dohoo et al., 2010b). If the test was significant ($P < 0.05$), the proportionality of each predictor was tested and the model was stratified on the non-proportional predictor. The model fit was evaluated graphically by assessing the proximity of the Cox-Snell residuals to having a unit exponential distribution (Dohoo et al., 2010b). Outliers were identified and evaluated using deviance residuals as well as score residuals. If outliers were found, they were explored to determine the characteristics of the observations that made them outliers and ensure data were not erroneous.

RESULTS

Descriptive Statistics

A total of 4,825 calves of unknown age were evaluated from November 2015 to September 2016 with 27% (n = 1,280), 28% (n = 1,368), 28% (n = 1,361), 9% (n = 447) and 8% (n = 369) entering Barns 1, 2, 3, 4 and 5, respectively. Most calves arrived during the summer (40%) with the fewest arriving during the fall (11%). Thirty percent of calves arrived in the spring and 21% of calves in the winter. The majority of the calves (70%) were drover-derived. Drovers 1, 2, 3, 4 and 5 were responsible for bringing 36%, 21%, 2%, 8% and 4% of the calves evaluated. The remaining calves were delivered directly by local farmers (18%) or purchased from auction markets (12%). The majority of the calves that arrived were male (99%). The females were retained in the analyses, as it is likely that they would have received similar treatment as the male calves on the source dairy farms, because the majority were free-martins. In addition, there was no difference in early ($P = 0.45$) or late mortality ($P = 0.77$) models when comparing male to female calves in univariable analyses.

The mean arrival weight of the calves was 47 kg (SD: 5 kg) with a range of 28 kg to 71 kg. Slaughter records existed for 4,041 calves and these calves were slaughtered for white veal after a mean growing period of 148 days (SD: 9 days). The mean hot carcass weight of the slaughtered calves was 127 kg (SD: 18 kg).

Health Parameters

Table 4.1 describes the proportion of calves with specific risk factors. Many calves entered the facility with some level of dehydration and low body condition. Of the dehydrated calves entering the facility, only 17% had diarrhea. Roughly a quarter of calves entered with an abnormal navel score (score of 2 and 3). Very few calves had abnormal respiratory or joint

scores. The mean rectal temperature was 39.2°C and 5% (n = 232) had a temperature ≥ 40°C. Observer 1 evaluated 35% of calves, whereas observer 2 and 3 evaluated 45% and 20%, respectively. Intra and inter-observer reliability were evaluated between observer 1 and 2 (Table 4.2). The calculated *K*appa statistics ranged from no to perfect agreement.

Mortality

Of the calves evaluated, 357 died (7%) of which 148 died in the first 21 days (42%) and 209 died after the first 21 days (59%). The Kaplan-Meier survival estimate (Figure 4.2) demonstrates the pattern of mortality is similar to what has been previously described (Bahler et al., 2012; Pardon et al., 2012a; Winder et al., 2016). Barn 1, 2, 3, 4, and 5 had a mortality risk of 8%, 10%, 6%, 6%, and 5%, respectively. The winter season had the highest overall mortality risk (11%) and fall had the lowest overall mortality (4%). Spring and summer had mortality risks of 8% and 6%, respectively.

Early Mortality Model

Based on the Youden Index, rectal temperature was cut at 39.2°C (Sensitivity (Se): 0.50; Specificity (Sp): 0.49; Area under the curve (AUC): 0.49). The mean time to mortality in the early mortality dataset was 10.54 days (SD: 5.31 days). The variables unconditionally associated with early mortality (≤ 21days following arrival) were barn, season, cough score, fecal score, navel score, dehydration score, sunken flank, source, and weight.

The final multivariable model contained 8 significant variables (Table 4.3). A navel score of 3, or elevated dehydration score were associated with higher hazard of early mortality. Drover-derived calves had a lower hazard of early mortality when compared to locally-derived calves; however, when drover-derived calves had a sunken flank, they had higher hazard of early mortality when compared to locally-derived calves without a sunken flank. Calves that weighed more on arrival also had lower hazard of early mortality.

Late Mortality Model

Based on the Youden Index, rectal temperature was cut at 39.1°C (Sensitivity (Se): 0.61; Specificity (Sp): 0.39; Area under the curve (AUC): 0.50) and body weight at arrival was cut at 48 kg (Sensitivity (Se): 0.41; Specificity (Sp): 0.64; Area under the curve (AUC): 0.52). The mean time to mortality in the late mortality dataset was 88.04 days (SD: 45.47 days). The initial Cox proportional hazard model did not meet the assumption of proportional hazards and the

following models were stratified based on season at arrival. The variables that were unconditionally associated with late mortality (> 21 days following arrival) were barn, nose score, eye score, cough score, navel score, and source.

In the final multivariable model, 3 variables were retained (Table 4.4). Being housed in barn 2, and a navel score of 3 were associated with higher hazard of late mortality. Being housed in barn 3 was associated with lower hazard of late mortality. Source of the calves was retained in the model as the overall variable had a significant P-value ($P = 0.02$) where calves being source from auction facilities had a trend for a higher hazard of late mortality.

DISCUSSION

This study demonstrates that abnormal navel, very low body condition score and sunken flank were common problems found at arrival. It also identified several health variables associated with mortality at the veal facility. One of the potential limitations of this study is the subjective nature of the scoring system used and the multiple observers that scored the calves. The Kappa statistics generated for ear, cough and dehydration score yielded slight to no agreement (Viera and Garrett, 2005) between observer 1 and 2. This is likely the result of the Kappa paradox, where despite a high percentage of inter-observer agreement, the corresponding value of Kappa may be relatively low (Feinstein and Cicchetti, 1990) because there is little variation in the condition being evaluated. The Kappa paradox may have occurred as the prevalence of those conditions was low in the group of calves scored for the calculation of Kappa (Dohoo et al., 2010c; Feinstein and Cicchetti, 1990; Viera and Garrett, 2005). The low number of calves examined for the calculation of the Kappa statistic (Sim and Wright, 2005) and the inability to examine the intra- and inter-observer reliability for observer 3 are further limitations that need to be considered.

Navel infections have been shown to have a significant impact on mortality (Donovan et al., 1998) and the overall health of the calf (Mee, 2008). Therefore, it is not surprising that arriving at the facility with a significantly enlarged navel, with heat, pain or malodorous discharge, was associated with both early and late mortality. The prevalence of abnormal navels was similar to what has been reported in other studies evaluating male calves (Bahler et al., 2012; Wilson et al., 2000). Navel infections cause not only a localized infection, but may spread by hematogenous dissemination and impact multiple organs (Wieland et al., 2017). Antibiotic treatment of the calves with navel infections at arrival could help to reduce mortality, however,

prevention on the source dairy farm would be preferable. Some preventative strategies that could be implemented at the source dairy farms include maintaining maternity pen hygiene, and ensuring adequate early intake of high quality colostrum (Mee, 2008). In terms of navel dipping, authors have reported differences with respect to certain types of navel dip (Robinson et al., 2015; Weiland et al., 2017). However, the paucity in the available literature that used a negative control group, makes it difficult to support the use of navel-dipping.

The severity of dehydration was a significant predictor of early mortality. Dehydration may arise from a variety of different factors including transportation and diarrhea. Transportation of young calves long distances can lead to a measurable increase in dehydration (Knowles et al., 1997). An electrolyte feeding during transit will reduce the extent of dehydration on arrival (Knowles et al., 1999) and could reduce early mortality. However, practically, this strategy may be difficult to implement. Diarrhea may also have been responsible for creating a dehydrated state (Smith, 2009b). Correction of dehydration in calves suffering from diarrhea is important in preventing mortality (Berchtold, 2009). However, fecal score was not significant in the final multivariable model evaluating early mortality and did not interact with or confound dehydration score, so it may not influence the role dehydration plays in early mortality. As many calves entered the facility with some degree of dehydration, measures to prevent and treat dehydration at arrival need to be explored to reduce mortality.

The presence of a sunken flank may be an indicator of the timing of the last milk or electrolyte feeding. Drover-derived calves were likely more susceptible to early mortality when a sunken flank was detected as the combination of increased energy demands from longer transportation (Knowles et al., 1997) and little to no energy input would lead to reduced disease resistance (Chandra, 1997; Godden et al., 2005; Ollivett et al., 2012). The presence of a sunken flank was also shown to impact mortality by Bahler et al. (2012), however, it was only significant in unconditional analysis. Thus, strategies employed to reduce time between meals for young calves, particularly when subjected to long transit times, could aid in reducing the risk of early mortality.

As previously demonstrated by others (Lava et al., 2016; Winder et al., 2016), barn number was associated with mortality. Brscic et al. (2012) and Lava et al. (2016) identified that housing, management and feeding practices are risk factors that can influence calf health and need to be considered at the barn level. As the barns in this study had different housing styles,

barn staff and feeding equipment, this could explain some of the variability in the mortality between the different barns.

Weight at arrival impacted early mortality. This finding is similar to other studies; where lower weight at arrival was associated with higher morbidity and mortality (Brscic et al., 2012; Winder et al., 2016). As previously suggested by Winder et al. (2016), weight at arrival may be a marker of age, nutrition and weight at birth. Knowles et al. (1995) described a strong negative correlation between age at transport and mortality. However, it is unclear whether the association with mortality is due to increased disease pressure occurring in the first weeks of life (Wells et al., 1997) or due to transportation. The topic of age at transportation requires further exploration, as a delay in transport could be explored as a method to reduce morbidity and mortality on veal farms.

Enhanced early life nutrition increases the growth of the calf (Borderas et al., 2009; Terre et al., 2006). It also improves disease resistance (Khan et al., 2007; Ollivett et al., 2012; Todd et al., 2017) and reduces mortality (Williams et al., 1981) due to more energy and protein being available to support immune function (Chandra, 1997). As heavier calves had a lower risk of early mortality, enhancing the nutrition of calves prior to arrival at the veal facility may provide an opportunity to improve disease resistance and lower mortality.

A surprising finding in this study was the high level of emaciation identified at arrival. This is also likely a reflection of nutritional status of the calf prior to arrival. Previous disease will also reduce average daily gain (Windeyer et al., 2014; Virtala et al., 1996) and impact body weight (Stanton et al., 2010) but could reduce body condition score. With 18% of calves arriving at the facility in an emaciated state, factors impacting body condition score should be explored and improved.

The source of the calves played a role in early and late mortality. Locally-derived calves had higher hazard of early mortality than those from drovers despite drover-derived calves likely having longer transportation times. Drovers could have implemented some type of screening prior to agreeing to transport calves, as they are penalized monetarily for light or sick calves at the veal facility. This screening would create an additional barrier of entry to the veal facility whereby calves that are ill and of low weight are not taken from the source farm thereby reducing the odds of mortality at the veal facility. Auction-derived calves had a trend for a higher hazard of late mortality. As auction calves are exposed to at least two different vehicles for

transportation and at least one temporary residence where they are commingled with many other calves, it creates an opportunity for the calf to be exposed to a variety of pathogens. As the auction environment contributes to mortality caused by bovine respiratory disease in veal calves (Palechek et al., 1987) and with the majority of deaths after 21 days resulting from pneumonia, this may be a plausible explanation for the association. Thus, strategies employed to optimize the conditions of purchase may lead to a reduction in the level of disease risk and in antimicrobial use (Lava et al., 2016).

Arrival at the facility in the summer was associated with early mortality. Higher early mortality during the summer differs from other studies evaluating female (Gulliksen et al., 2009; Lombard et al., 2007) and male calf mortality (Winder et al., 2016). However, heat stress occurring pre- and post-partum can affect immune responses in calves (Tao and Dahl, 2013; Roland et al., 2016) making them more susceptible to disease. The lack of heat dissipation strategies employed in the barns combined with one of the hottest summers recorded in southwestern Ontario (Seglenieks, 2016) may have contributed to an increased early mortality in the summer. Season at arrival did not meet the assumption of proportional hazards in the late mortality suggesting that season has a differing effect on the hazard of mortality over time specifically during the later portion of the growing period.

The presence induced repeated or spontaneous coughs can be used to identify calves with early respiratory disease (Poulsen and McGuirk, 2009). Coughing is an important respiratory defense mechanism that is initiated by irritation of receptors in the airways (Smith, 2009a). With repeated or spontaneous coughing being a hallmark clinical sign of acute or chronic respiratory disease (Andrews, 2004), interventions should be explored to reduce mortality levels.

CONCLUSIONS

The prevalence of health problems at arrival represents important risks for mortality in veal calves. Many calves at high risk for mortality can be identified upon arrival. Intervention on high-risk calves may be a strategy to reduce the risk of mortality, however preventative measures applied prior to arrival at the veal facility would be a preferred approach.

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Table 4. 1. Description and prevalence of risk factors scored on 4,825 calves on arrival at a milk-fed veal facility.

Variable		Score				
		0	1	2	3	4
Nose Score	Description	Normal serous discharge	Small amount of unilateral discharge	Bilateral, cloudy or excessive discharge	Copious, bilateral mucopurulent discharge	
	Prevalence % (n)	75.1 (3,722)	23.2 (1,151)	1.6 (80)	0 (3)	
Eye Score	Description	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge	
	Prevalence % (n)	67.3 (3,335)	30.4 (1,505)	2.2 (108)	0.1 (6)	
Ear Score	Description	Normal	Ear flicking	Slight unilateral droop	Head tilt or bilateral droop	
	Prevalence % (n)	92.8 (4,589)	6.6 (327)	0.5 (25)	0 (2)	
Cough Score	Description	No cough	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous cough	
	Prevalence % (n)	94.7 (4,676)	4.1 (203)	1.2 (58)	0.1 (3)	
Fecal Score	Description	Normal	Semi-formed, pasty	Loose, stays on top of bedding	Watery, sifts through bedding	
	Prevalence % (n)	65.8 (3,257)	21.3 (1,055)	8.8 (435)	4.1 (204)	

Navel Score	Description	Normal	Slightly enlarged, not warm or painful	Slightly enlarged with slight pain or moisture	Enlarged with heat, pain or malodorous discharge
	Prevalence % (n)	26.5 (1,311)	47.6 (2,360)	19.7 (978)	6.2 (307)
Joint Score	Description	Normal	Slight swelling, not warm or painful	Swelling with pain or heat, slight lameness	Swelling with severe pain, heat and lameness
	Prevalence % (n)	98.8 (4,870)	0.9 (44)	0.2 (9)	0.1 (6)

Dehydration Score	Description	Skin tent returns to normal <2 seconds, bright alert, strong suckle ($\leq$ 5% dehydrated)	Skin tent returns to normal in 2 seconds, eyes not sunken, good suckle (6 to 8% dehydrated)	Good suckle, eyes slightly sunken, skin tent returns to normal in 2-4 seconds (8 to 10% dehydrated)	Mild depression, sternal recumbency, moderately sunken eyes, skin tent returns to normal in 4-8 seconds, tacky mucus membranes with poor suckle (10 to 12% dehydrated)	Profound depression, absent suckle, lateral recumbency, eyes deeply sunken, skin tent returns to normal in >8-10 seconds. Dry mucous membranes. (>12% dehydrated)
	Prevalence % (n)	53.8 (2,648)	34.6 (1,701)	10.2 (504)	1.4 (67)	0.1 (3)
BCS	Description	Subcutaneous fat covering bony prominences	Thin covering of subcutaneous fat over bony prominences	Bony prominences are easily palpated	No subcutaneous fat covering frame	Emaciated with little muscle or fat present and clearly defined bone structure
	Prevalence % (n)	1.4 (70)	7.7 (378)	40.2 (1,981)	32.7 (1,609)	18.0 (887)
Sunken Flank	Description	No	Yes			
	Prevalence % (n)	79.9 (3,951)	20.1 (991)			

Table 4. 2. Inter- and intra-observer agreement between observer 1 and 2 for risk factors evaluated at arrival at a milk-fed veal facility n = 25 calves

Variable	Intra-observer agreement		Inter-observer agreement	
	% agreement	Weighted Kappa[1]	% agreement	Weighted Kappa[1]
Nose Score	97	0.50	97	0.38
Eye Score	97	0.65	97	0.67
Ear Score	100	1.00	96	0.00
Cough Score	94	0	91	-0.05
Navel Score	95	0.75	93	0.69
Joint Score	100	1.00	100	1.00
Dehydration Score	80	0.27	71	0.08
BCS	90	0.53	89	0.31

[1]Calculated as described by Cohen (1968)

Table 4. 3. Results from a multivariable Cox proportional hazard model of early mortality (≤ 21 days after arrival) at the veal facility n = 4,689 calves with 143 mortalities

Variable	Description	n	Mortality Risk (%)	Hazard Ratio	95% Confidence Interval	P
Barn	1	1,212	3.55	Ref.		
	2	1,339	2.91	0.84	0.54 to 1.32	0.45
	3	1,342	3.5	1.04	0.67 to 1.60	0.86
	4	440	2.27	0.65	0.32 to 1.32	0.24
	5	356	1.12	0.35	0.12 to 1.00	0.05
Season	Winter	987	3.65	Ref.		
	Spring	1,357	2.58	1.03	0.63 to 1.70	0.91
	Summer	1,855	3.45	1.99	1.17 to 3.38	0.01
	Fall	490	1.63	0.79	0.35 to 1.79	0.58
Navel score	0 and 1	3,467	2.74	Ref.		
	2	928	3.34	1.26	0.84 to 1.90	0.27
	3	294	5.78	2.40	1.42 to 4.07	<0.01
Cough score	0	4,441	2.88	Ref.		
	1	188	5.32	1.62	0.84 to 3.11	0.15
	2 and 3	60	8.33	3.13	1.25 to 7.84	0.02
Dehydration score	0	2,553	2.27	Ref.		
	1	1,609	3.48	1.75	1.18 to 2.60	0.01
	2	465	4.73	2.04	1.18 to 3.53	0.01
	3 and 4	62	11.29	6.73	2.83 to 16.00	<0.01
Sunken Flank	No	3,768	2.76	Ref.		
	Yes	921	4.23	0.67	0.23 to 1.96	0.47
Source	Local	844	4.62	Ref.		
	Drover 1	1,711	2.81	0.41	0.25 to 0.67	<0.01
	Drover 2	994	2.21	0.32	0.17 to 0,62	<0.01
	Drover 3	78	2.56	0.30	0.04 to 2.20	0.24
	Drover 4	357	1.68	0.25	0.10 to 0.60	<0.01
	Drover 5	168	4.17	0.45	0.14 to 1.48	0.19
	Auction	537	3.05	0.79	0.43 to 1.44	0.44
Weight	Per 1 kg increase			0.93	0.89 to 0.97	<0.01
Interaction Term Sunken Flank X Source	No X Local			Ref.		
	Yes X Drover 1			3.63	1.10 to 11.99	0.04
	Yes X Drover 2			3.64	0.92 to 14.42	0.07
	Yes X Drover 5			5.80	0.93 to 36.28	0.06

Table 4. 4. Results from a multivariable Cox proportional hazard model of late mortality (>21 days after arrival) at the veal facility stratified by season at arrival n = 4,610 calves with 204 mortalities

Variable	Description	n	Mortality Risk (%)	Hazard Ratio	95% Confidence Interval	P
Barn	1	1,201	4.25	Ref.		
	2	1,308	6.73	1.44	1.01 to 2.05	0.04
	3	1,305	2.91	0.64	0.42 to 0.98	0.04
	4	436	3.44	0.80	0.44 to 1.44	0.45
	5	366	3.33	0.83	0.43 to 1.62	0.59
Source	Local	815	4.17	Ref.		
	Drover 1	1,670	4.61	1.08	0.72 to 1.62	0.70
	Drover 2	972	3.7	0.76	0.48 to 1.22	0.26
	Drover 3	78	6.41	1.79	0.70 to 4.59	0.23
	Drover 4	356	1.68	0.56	0.23 to 1.36	0.20
	Drover 5	161	4.34	0.78	0.34 to 1.77	0.55
	Auction	527	7.4	1.56	0.98 to 2.48	0.06
Navel Score	0 and 1	3,422	4.12	Ref.		
	2	910	4.62	1.14	0.81 to 1.60	0.46
	3	278	7.55	1.80	1.13 to 2.86	0.01

Figure 4. 1. Causal diagram describing the relationship of measured variables to mortality occurring during the growing period at a milk-fed veal calf facility in Ontario.
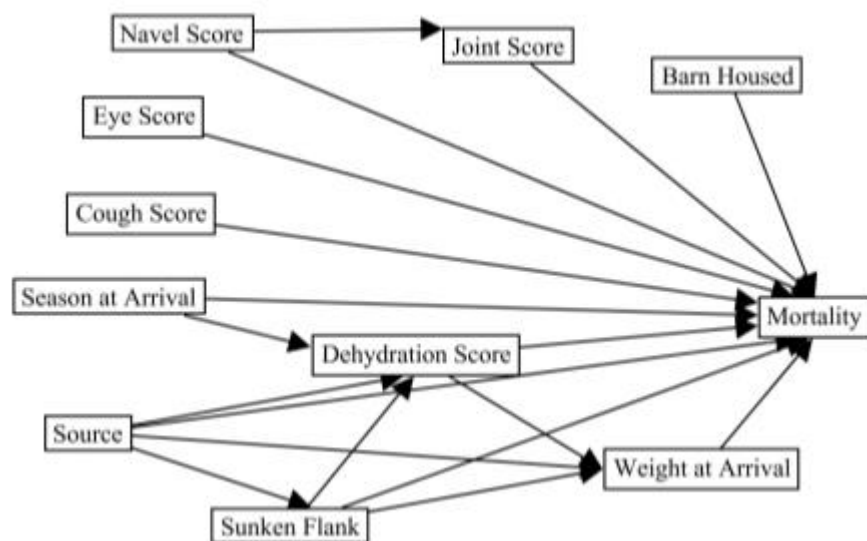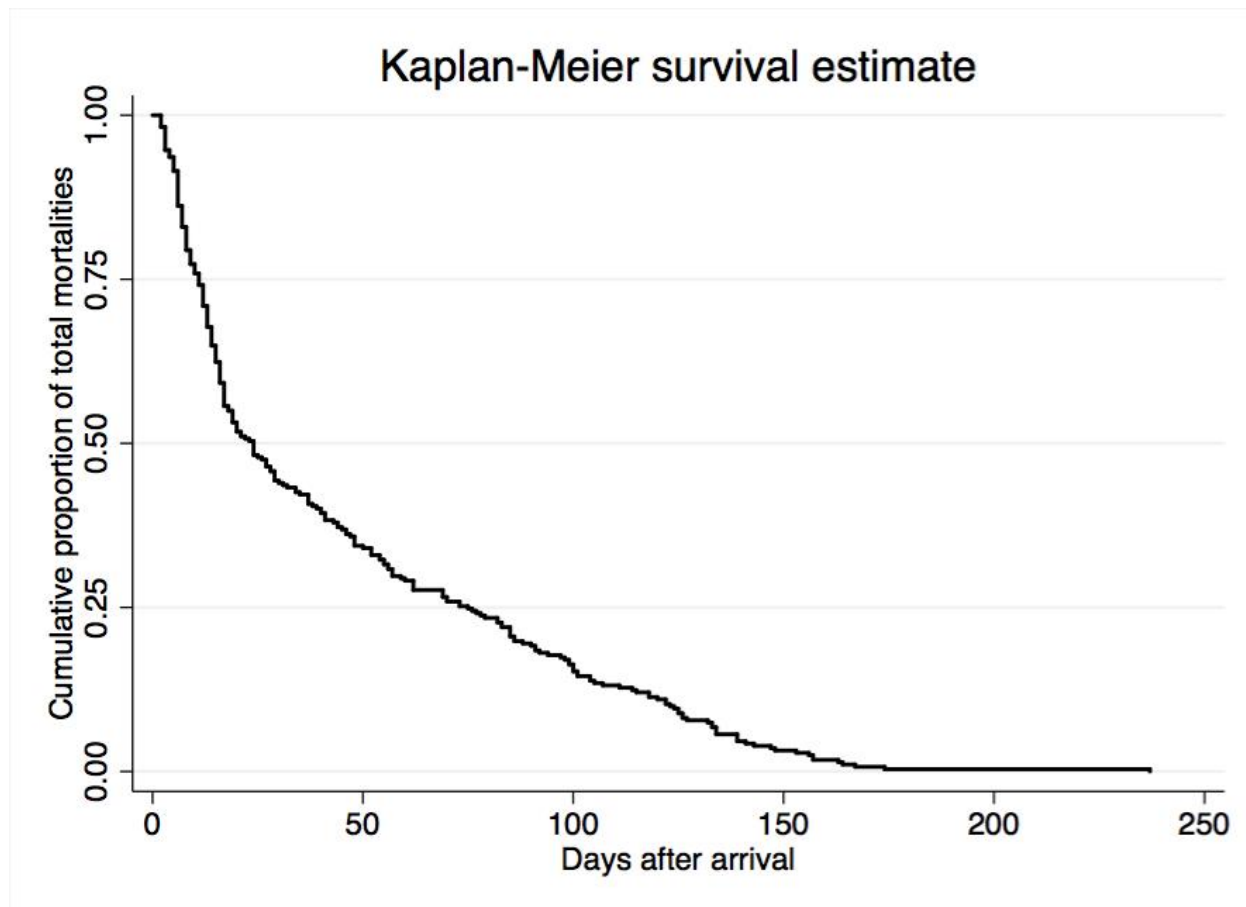
Figure 4. 2. Kaplan-Meier survival estimate; days after arrival at which death occurred in 347 calves screened at arrival to a milk-fed veal facility

CHAPTER 5: CLINICAL AND METABOLIC INDICATORS ASSOCIATED WITH EARLY MORTALITY AT A MILK-FED VEAL FACILITY: A PROSPECTIVE CASE-CONTROL STUDY

ABSTRACT

Antimicrobial use and resistance in combination with high levels of mortality are important challenges that the veal industry faces. In order to improve both the economic sustainability of the industry and animal welfare, measures need to be taken to explore and address reasons for these challenges. Health status at arrival may be an important predictor of calf mortality as substantial mortality occurs early in the growing period on veal operations. The objective of this observational case-control study was to identify clinically measurable variables and metabolic indicators associated with mortality in the first 21 days following arrival at a veal facility. Calves were evaluated using a standardized health scoring system, blood was collected, weight was measured and the supplier of the calf was recorded at arrival. The calves were followed until death or 21 days after arrival. Cases were defined as calves that died $\leq$ 21 days following arrival. Two controls for every case were randomly selected from calves that survived > 21 days, arrived on the same day and were housed in the same barn as cases. Stored serum harvested at arrival from cases and controls was submitted for measurement of concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyrate acid (BHBA), glucose, cholesterol, urea, haptoblobin and immunoglobulin G (IgG). A conditional logistic regression model was built to evaluate factors associated with mortality $\leq$ 21 days following arrival. A total of 4,825 calves were evaluated from November 2015 to September 2016. The mortality risk in the first 21 days was 2.8% giving 135 cases which were compared with 270 controls. There were 6 variables that were significant in the final multivariable model. Calves with a slightly enlarged navel with slight pain or moisture, and those with severe dehydration had increased odds of mortality $\leq$ 21 days following arrival. Drover-derived calves, calves that weighed more, or had higher concentration of IgG or cholesterol at arrival were less likely to die. The results demonstrate that calves at elevated risk for early mortality can be identified at arrival using both health and hematological factors. Early recognition of high-risk calves may allow for an intervention that could result in improvement in survival rates, however, prevention of these abnormalities prior to arrival at veal facilities needs to be further explored.

INTRODUCTION

Calf morbidity and mortality represent a significant cost to calf-rearing industries (Nor et al., 2012) and an important concern for animal welfare (Ortiz-Pelaez et al., 2008). With mortality ranging from 5% to 8% in conventional veal housing (Pardon et al., 2012a; Winder et al., 2016) and 4% in animal welfare specific housing (Bahler et al., 2012; Lava et al., 2016), there is a clear need to address its occurrence. The intensive use of antimicrobials is another important challenge faced by the veal industry (Pardon et al., 2014). In Europe, the veal calf sector uses high levels of antimicrobials (Bos et al., 2013; Lava et al., 2016; Pardon et al., 2012b), however, in Canada and the United States the amounts used are unknown. The level of antibiotic use in the veal industry has been associated with the emergence of antimicrobial resistance in commensal, pathogenic, and zoonotic bacteria (Catry et al., 2007; Catry et al., 2016; Cook et al., 2011). This highlights the urgent need for the veal industry to change (Murphy et al., 2016) and for the industry to remain viable, controllable risk factors need to be identified and modified to decrease morbidity and mortality.

As most mortality occurs during the early portion of the growing period, this may provide an initial area of focus (Pardon et al., 2012a; Winder et al., 2016). Health status and weight at arrival at a veal facility can aid in the prediction of mortality early in the growing period (Bahler et al., 2012; Renaud et al., 2017a; Winder et al., 2016). However, metabolic indicators may also play a role in identification of calves that are at increased risk of morbidity or mortality.

Colostrum management could be a key factor contributing to calf losses in male calf rearing (Godden, 2008). Immunoglobulin G (IgG) and total protein can both be used as markers for colostrum intake, with IgG being more specific in identifying failure of passive transfer (Weaver et al., 2000). Currently, the only tests that directly measure serum IgG are the enzyme linked immunosorbent assay (ELISA) and radial immunodiffusion (Weaver et al, 2000).

However, given the semi quantitative nature of the ELISA, radial immunodiffusion is used as the gold standard test (Fecteau et al., 2013). Failure of passive transfer is a common problem in male calves (Pardon et al., 2015; Trotz-Williams et al., 2008) and a strong association between serum IgG concentration and morbidity in male calves has been found (Pardon et al., 2015). However, a clear link between IgG status and mortality has not yet been established in the veal industry.

The acute phase response is a non-specific reaction that occurs in response to tissue injury and leads to the production of acute phase proteins. Haptoglobin is an acute phase protein that increases in serum during bacterial and viral disease (Ganheim et al., 2007). It has been used to identify calves with pneumonia (Angen et al., 2009) and as a prognostic tool for calves with diarrhea (Hajimohammadi et al., 2011). Alpha-2 globulins levels at arrival at a veal facility, of which haptoglobulin is a fraction, has also been shown to impact neonatal calf diarrhea and average daily gain (Pardon et al., 2015). As haptoglobin concentration is low in healthy calves (Ganheim et al., 2003), it could be used as a screening tool to identify diseased calves soon after arrival at a veal facility.

Improved energy status protects against disease and supports immune function (Todd et al., 2017). With energy expenditure and mobilization occurring during calf transport (Knowles et al., 1999), combined with 17% of surveyed Canadian dairy source farms providing inferior nutrition to male calves compared to female calves (Renaud et al., 2017b), many male calves enter the veal industry with sub-optimal energy status and low body fat cover (Wilson et al., 2000). Serum concentration of beta-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), cholesterol, glucose and urea could all serve as markers of energy status in calves.

The objective of this study was to identify clinically measurable health and metabolic indicators associated with mortality occurring in the first 21 days following arrival at a veal facility.

MATERIALS AND METHODS

This observational case-control study was conducted in cooperation with a single milk-fed veal calf producer and in accordance with the University of Guelph Animal Care Committee (Animal Use Protocol: #3453). The producer had 5 barns in different geographical locations in south-western Ontario, Canada. The diet provided to the calves did not differ by barn, however, there were several management differences. Barns 1, 2 and 4 fed calves manually, whereas barns 3 and 5 used automated calf feeders. Calves were housed individually in barns 1 and 4 and in groups of 60 calves in barns 3 and 5. Barn 2 housed calves in individual pens in early life, transitioning to groups of 8 calves at 5 to 6 weeks following arrival.

Data Collection

When calves arrived at the receiving facility, they were immediately evaluated using a standardized health scoring system and weighed with a digital weighing scale (Cardinal Scale Manufacturing Co.; Webb City, MO). The supplier of the calf and arrival date were also recorded. The suppliers were placed into 3 categories; local, drover and auction. 'Local' refers to dairy farmers who delivered calves directly to the veal facility. 'Drover' was used for calves that were transported from multiple dairy farms to the veal facility and 'auction' were calves derived from auction facilities. Calves were identified on arrival based on their Canadian Cattle Identification Agency ear tag using a hand-held RFID reader. Mortalities occurring during the growing period were recorded using an electronic recording database (Trax-IT©; Merit-Trax Technologies; Mount Royal, Quebec, Canada).

Standard Health Scoring System

An iPad (Apple Inc.; Cupertino, CA) with the Calf Health Scorer app (University of Wisconsin-Madison, Madison, WI) and Qualtrics[TM](http://www.qualtrics.com/) was used to facilitate the health scoring. The Calf Health Scorer provided images and descriptions to evaluate the respiratory system (nose, eye, ear, cough) (McGuirk and Peek, 2014), fecal consistency (McGuirk, 2008), navel inflammation (adapted from Fecteau et al., 1997), joint swelling, and rectal temperature. A Qualtrics[TM] form was used to collect data on the evaluation of dehydration (Wilson et al., 2000), body condition score (BCS) (Wilson et al., 2000) and sunken flank (Bähler et al., 2012) (Table 5.1). All calves were examined by one of three different observers; however, all case and control pairs were evaluated by the same observer.

Blood Collection and Processing

Following the health examination, approximately 10 ml of whole blood was collected from the jugular vein into a sterile blood collection tube without an anticoagulant (BD Vacutainer; Becton, Dickson and Co., Franklin Lakes, NJ, USA). Blood was transported on ice to the laboratory where it was allowed to clot and then centrifuged at $1,500 \times g$ for 15 minutes at approximately 20°C. The approximate time from blood collection until centrifugation of the blood was 3 hours. Serum was separated and stored at -20°C until submission to the Animal Health Laboratory (Guelph, ON, Canada) and Saskatoon Colostrum Company (Saskatoon, SK, Canada) for further analysis. Serum from cases and controls was analyzed for non-esterified fatty acids (NEFA), beta-hydroxybutyrate acid (BHBA), glucose, cholesterol, urea, haptoblobin and immunoglobulin G (IgG). The biochemistry testing was done on the Roche Cobas 6000 c501 automated chemistry analyzer (Roche Canada, Laval, QC, Canada). NEFA and BHBA concentrations were measured using Randox NEFA and Randox BHBA kits (Randox Laboratories Canada Ltd., Mississauga, ON, Canada). Glucose concentration was determined using the Roche GLU3 kit (Roche Canada, Laval, QC, Canada) whereas cholesterol concentration was determined using the Roche CHOL2 kit (Roche Canada, Laval, QC, Canada). The Roche UREAL kit (Roche Canada, Laval, QC, Canada) was used to measure urea concentrations. Haptoglobin concentrations were measured by determining the hemoglobin binding capacity of serum and quantified against a standard sample (Skinner et al., 1991). Serum IgG was determined by radial immunodiffusion as described by Chelack et al. (1993).

Selection of Cases and Controls

A calf was selected as a case if the calf died ≤ 21 days after arriving at the facility. This cut point was selected based on previous work that demonstrated that a significant proportion of mortality occurred in the first 3 weeks after arrival at veal calf facilities (Bahler et al., 2012; Pardon et al., 2012a; Winder et al., 2016). Two controls for each case were randomly selected from calves that survived > 21 days, arrived on the same day and were housed in the same barn as the cases. Two controls were used to improve the precision of the association estimates (Dohoo et al., 2010a). All calves selected were male.

Statistical Analysis

All statistical analyses were completed using Stata 14 (StataCorp LP, College Station, TX). Data were imported from Microsoft Excel (Microsoft®; Redmond, WA) into Stata 14 and

checked for completeness. Calves with missing data were deleted from analysis (complete-case analysis; Pigott, 2001). A causal diagram was created to evaluate the relationship between mortality and the measured variables (Figure 5.1). Descriptive statistics were generated for all explanatory variables in the dataset.

Blood measures of cases and controls were summarized to describe characteristics of both groups. A Student's t-test was used to identify significant ($P \leq 0.05$) differences between cases and controls for normally distributed parameters and a Kolmogorov-Smirnow test was used for non-normally distributed parameters. All blood parameters had normal distributions except BHBA, haptoglobin, and urea.

A single conditional logistic regression model was built to explore associations with mortality ≤ 21 days following arrival. The assumption of linearity of continuous predictor variables was assessed by plotting the logarithmic odds of the outcome against the variable. If a variable failed to meet the linearity assumption, the variable was categorized into 2 categories. NEFA, haptoglobin and BHBA were categorized based on cut points generated by the Youden Index (Youden, 1950). The Youden Index is a summary measure of the receiver operator curve (ROC), measuring the accuracy of a diagnostic marker and generating an optimal cut-point for the marker (Fluss et al., 2005). Co-linearity among the explanatory variables was tested using Spearman rank coefficients. If the correlation coefficient between 2 variables was ≥ 0.6, only one variable was retained based on fewest missing values, reliability of measurement and/or biological plausibility. Univariable logistic regression models were constructed to identify variables that were unconditionally associated with the outcome using a *P*-value of 0.2. Risk factors that had univariate associations ($P < 0.2$), were subsequently offered to a multivariable model through a manual backward stepwise process. Variables were retained in the multivariable models if $P < 0.05$. Evaluating the effect of the removed variables on the coefficients of the remaining variables was used to assess confounding. A variable was deemed to be a confounder if it was not an intervening variable based on the causal diagram and the log odds of a significant variable in the model changed by at least 20%. Two-way interactions were evaluated between variables suspected to interact based on evidence from the literature and remained in the final model if significant ($P < 0.05$) (Dohoo et al., 2010b). Outliers were identified and evaluated using Pearson residuals and deviance residuals as well as delta-betas, case-control group delta-betas, delta-χ^2 and case-control group delta-χ^2 (Dohoo et al., 2010c). If outliers were found, they

were explored to determine the characteristics of the observations that made them outliers. The outliers were retained in the analysis unless the magnitude and direction of the coefficients in the final model were altered by the removal of the data points or if the data was found to be erroneous.

For continuous metabolic indicators in blood that were significant in the final multivariable model, and linearly associated with logarithmic odds of the outcome, a Youden's Index (Youden, 1950) was used to determine cut points.

RESULTS

Descriptive Statistics

A total of 4,825 calves were evaluated from November 2015 to September 2016. There were 135 cases that died $\leq$ 21 days following arrival, representing a mortality risk of 2.8%. A total of 270 controls were randomly selected using the criteria previously described. The majority (43%) of case and control calves arrived at the facility in the summer months (June to August) with 26%, 25% and 6% arriving in the spring (March to May), winter (December to February) and Fall (September to November), respectively. The calves were assigned to 5 different barns based on the availability of rooms within the barn. Barn 1, 2, 3, 4 and 5 housed 27%, 28%, 35%, 7%, 4% of the calves, respectively. The calves were derived from 120 sources which were categorized into three main groups. Local, drover and auction-derived calves represented 21%, 66% and 13% of the overall population, respectively.

Health Parameters

Table 2 describes the proportion of cases and controls with specific health attributes. A chi-square value was calculated for each health attribute to identify statistically significant differences between the case and control groups (Table 2). Dehydration and emaciation were present in 50% and 54% of calves at arrival, respectively. Roughly a quarter of calves entered the facility with an abnormal navel score or fecal score. A calf seldom entered the facility with an abnormality in the respiratory or joint parameters. Rectal temperature (Table 3) did not differ numerically between cases and controls. Three observers evaluated all the calves upon arrival with observer 1, 2 and 3 examining 40%, 45% and 15% of the case control groups, respectively.

Blood Parameters

There were significant differences between cases and controls in weight at arrival, and serum BHBA, glucose, cholesterol and IgG (Table 3). A total of 14 (10%) cases and 6 (2%)

controls were hypoglycemic (< 3.3 mmol/L (Smith, 2009)). Based on the Youden Index, haptoglobin was cut at 0.18 g/L (Sensitivity (Se): 0.67; Specificity (Sp): 0.39; Area under the curve (AUC): 0.51), NEFA was cut at 0.35 mmol/L (Se: 0.67; Sp: 0.39; AUC: 0.53), and BHBA was cut at 60.50 μmol/L (Se: 0.34; Sp: 0.55; AUC: 0.45).

Early Mortality Model

The variables unconditionally associated with early mortality are found in Table 5.4. In the final multivariable model, 6 variables were significant (Table 5.5). A navel score of 2 and a dehydration score of 4 and 5 were associated with higher odds of mortality. Drover-derived calves, greater weight at arrival, greater concentrations of IgG or cholesterol were associated with lower odds of mortality. No interactions were identified in the final model. A single match group outlier was identified; however, it was not determined to be a recording error and it was retained in the final model as the magnitude and direction of the coefficients did not change.

Cut points generated by Youden's Index for continuous variables that were significant in the final model and linearly associated with logarithmic odds of the outcome are presented in Table 6.

DISCUSSION

This study demonstrated that low serum IgG and cholesterol concentrations are associated with increased risk for mortality in the first 21 days following arrival at a veal facility. To our knowledge, this is the first study to identify cholesterol as a marker for mortality in calves arriving at a veal facility. However, creating cut points for IgG and cholesterol yielded poor estimates of sensitivity and specificity, suggesting that, as a stand-alone test, both parameters are poor at identifying calves at high-risk for early mortality and it may not be economical to use these tests practically. Health status at arrival, specifically navel score and the degree of dehydration, were predictors of mortality occurring in the first 21 days following arrival. The source of the calves, and weight on arrival were associated with early mortality. A limitation to this study was the length in time from blood collection until serum separation. This would lead to an underestimation of glucose levels due to the utilization of glucose by red blood cells.

Navel score association with mortality is not a surprising finding as navel infection has been previously shown to impact mortality and the overall health of calves (Donovan et al., 1998; Mee, 2008). The high prevalence of calves arriving with abnormal navel score is similar to previous literature (Wilson et al., 2000) and demonstrates a clear need to further explore

preventative measures for this condition. Calves that were > 10% dehydrated at arrival were at increased risk of mortality. Dehydration is also a common issue found in the veal industry (Wilson et al., 2000) and could reflect the time in transit from the source dairy farm (Knowles et al., 1997). Weight at arrival has been previously demonstrated to impact both morbidity (Brscic et al., 2012) and mortality (Winder et al., 2016) in the veal industry. It is unclear whether weight is a reflection of age or nutritional status at the source dairy farm, but it needs to be explored as a mechanism to reduce the risk of mortality. Locally derived calves had an increased risk of mortality when compared to drover derived calves. As drovers were more likely to be economically penalized by the veal facility for calves in poor health, this may have caused drovers to implement a screening process to select healthier calves prior to transportation. For a more thorough discussion on the impact of health status, weight, and source of the calves on mortality see Renaud et al. (2017a).

Calves depend almost entirely on the absorption of maternal immunoglobulins from colostrum after birth to protect against common pathogens until the functional maturity of their own immune system develops (Godden, 2008). Thus, it is expected that the higher the concentration of IgG, the lower the risk of mortality. One of the major challenges with veal production is that veal producers rely on the dairy producers to provide the necessary care of these calves on the dairy farm of origin prior to departure. As a minority (9%) of Canadian dairy producers did not always feed colostrum to male calves (Renaud et al., 2017b), this is an area that needs to be addressed to improve the health and welfare of male calves.

Hypoglycemia is a common metabolic derangement occurring in neonatal calves (Smith, 2009). It can occur due to generalized infection (Lofstedt et al., 1999), diarrhea (Santos et al., 2002), and the withdrawal of milk (Smith, 2009). In this study, the prevalence of hypoglycemia was higher in cases when compared to controls and glucose concentrations were associated with mortality in the unconditional analysis. However, the relationship between hypoglycemia and mortality did not remain in the final regression model suggesting that other factors were more important for predicting mortality in this study. These findings in regards to glucose, need to be taken in light of the serum not being separated immediately.

There could be multiple explanations for the association of serum cholesterol with early mortality. Cholesterol deficiency haplotype (CDH), which results in low levels of cholesterol (Otter and Hately, 2017), is a cause of emaciation, growth retardation, and diarrhea, leading to

increased levels of mortality (Kipp et al., 2016). With the lineage of this deficiency tracing back to a prominent sire in Canada (Maughlin Storm) (Kipp et al., 2016), this haplotype may be common in Canada. Cholesterol could also be used as a marker of colostrum intake. Cholesterol concentration is much higher in colostrum than in milk and concentrations in the first days of life are proportional to the amounts of ingested colostrum (Ontsouka et al., 2016). Cholesterol in colostrum plays a critical role in mediating postnatal growth and development by influencing intestinal signaling and promotion of intestinal lactase activity, which may impact mortality and morbidity in calves (Ontsouka et al., 2016). Cholesterol concentrations in serum also increase with age (Piccione et al., 2010). As calves transported at older ages had lower mortality after transportation (Knowles et al., 1995), low cholesterol concentrations may represent younger calves. As multiple factors could influence cholesterol concentrations, a further understanding of the mechanisms surrounding cholesterol's influence on mortality need to be explored.

NEFA, BHBA and urea, which were used as markers of energy status, were not associated with mortality. Knowles et al. (1999) had previously found differences in long distance transport of calves with respect to these parameters. However, in this study, it is unlikely that calves were transported or held off feed for more than 10 hours and these effects may only have been present in calves transported for longer durations.

Haptoglobin was not a good indicator of early mortality when evaluated on arrival. Because acute phase proteins increase rapidly coinciding with the onset of clinical signs or subclinical inflammation, haptoglobin may not identify calves during a disease incubation period (Svensson et al., 2007). Haptoglobin also increases in response to stress of transportation (Lomborg et al., 2008), which may result in elevated haptoglobin concentrations. These reasons could explain the poor performance of haptoglobin in identifying individual calves at increased risk for disease or death. Similar conclusions were made by Svensson et al. (2007) and Murray et al. (2014) where haptoglobin had a poor discriminative ability to identify individual calves with or at risk for disease.

CONCLUSIONS

Assessing health and metabolic factors on arrival at a veal facility can identify calves at risk for mortality in the first 21 days after arrival. Calves with low levels of cholesterol and IgG at arrival are at greater risk for early mortality. Navel score, dehydration level, source and weight should also be evaluated to identify high-risk calves. Screening of calves at arrival to a veal

facility may allow for detection and intervention on high-risk calves to improve survival rates, however, emphasis should be placed on preventative measures, such as improved colostrum management and navel care, prior to arrival to the veal facility.

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Table 5. 1. Description of health variables scored on arrival to the milk fed veal facility.

Variable	Score				
	0	1	2	3	4
Nose Score	Normal serous discharge	Small amount unilateral discharge	Bilateral, cloudy or excessive discharge	Copious, bilateral mucopurulent discharge	
Eye Score	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge	
Ear Score	Normal	Ear flicking	Slight unilateral droop	Head tilt or bilateral droop	
Cough Score	No cough	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous cough	
Fecal Score	Normal	Semi-formed, pasty	Loose, stays on top of bedding	Watery, sifts through bedding	
Navel Score	Normal	Slightly enlarged, not warm or painful	Slightly enlarged with slight pain or moisture	Enlarged with heat, pain or malodorous discharge	
Joint Score	Normal	Slight swelling, not warm or painful	Swelling with pain or heat, slight lameness	Swelling with severe pain, heat and lameness	
Dehydration Score	Skin tent returns to normal <2 seconds, bright alert, strong suckle ($\leq$ 5 % dehydrated)	Skin tent returns to normal in 2 seconds, eyes not sunk, good suckle (6 to 8% dehydrated)	Good suckle, eyes slightly sunken, skin tent returns to normal in 2-4 seconds (8 to 10% dehydrated)	Mild depression, sternal recumbency, moderately sunken eyes, skin tent returns to normal in 4-8 seconds, tacky mucus membranes with poor suckle	Profound depression, absent suckle, lateral recumbency, eyes deeply sunken, skin tent returns to normal in >8-10 seconds. Dry

				(10 to 12% dehydrated)	mucous membranes. (> 12% dehydrated)
BCS	Subcutaneous fat covering bony prominences	Thin covering of subcutaneous fat over bony prominences	Bony prominences are easily palpated	No subcutaneous fat covering frame	Emaciated
Sunken Flank	No	Yes			

Table 5. 2. Frequency distribution of calf health attributes scored on arrival at a milk-fed veal facility for 135 calves that died ≤ 21 days following arrival to a milk-fed veal facility (case) and 270 calves that survived > 21 days following arrival to a milk-fed veal facility (control).

Variable		Score					Chi-Square
		0	1	2	3	4	*P*
Nasal Score	Case %	76	23	0	0	-	0.88
	Control %	76	24	0	0	-	
Eye Score	Case %	70	27	2	0	-	0.49
	Control %	65	33	2	0	-	
Cough Score	Case %	90	7	3	0	-	0.02
	Control %	94	6	0	0	-	
Fecal Score	Case %	61	19	13	7	-	0.18
	Control %	68	21	8	3	-	
Navel Score	Case %	19	47	23	11	-	0.17
	Control %	21	55	17	6	-	
Joint Score	Case %	98	1	0	2	-	0.05
	Control %	100	0	0	0	-	
BCS	Case %	1	7	41	35	16	0.78
	Control %	2	8	34	39	17	
Dehydration Score	Case %	41	39	15	5	0	0.01
	Control %	55	31	12	1	0	
Sunken Flank	Case %	73	27	-	-	-	0.30
	Control %	77	23	-	-	-	

Table 5. 3. Weight, rectal temperature and metabolic parameters for 135 calves that died $\leq$ 21 days following arrival to a milk-fed veal facility (case) and 270 calves that survived > 21 days following arrival to a milk-fed veal facility (control).

Variable	Group	Mean	SD[1]	Min	Max	*P*
Weight (lbs)	Case	99.84	10.44	80.0	129.0	0.02
	Control	102.62	11.27	75.0	151.0	
Rectal Temp. (°C)	Case	39.16	0.55	37.7	41.7	1.00
	Control	39.16	0.49	37.9	40.7	
BHBA (umol/L)	Case	62.24	54.72	0.0	299.0	0.003
	Control	77.58	75.54	2.0	661.0	
Cholesterol (mmol/L)	Case	1.44	0.49	0.2	2.7	<0.001
	Control	1.79	0.61	0.6	4.2	
Glucose (mmol/L)	Case	4.95	1.15	2.1	7.5	0.009
	Control	5.24	0.99	1.8	8.1	
Haptoglobin (g/L)	Case	0.29	0.38	0.1	2.5	0.50
	Control	0.23	0.29	0.1	3.3	
NEFA (mmol/L)	Case	0.41	0.16	0.1	0.9	0.28
	Control	0.43	0.18	0.1	1.0	
Urea (mmol/L)	Case	3.91	2.52	1.0	17.6	0.36
	Control	3.59	2.24	0.8	25.0	
IgG (g/L)	Case	13.6	9.89	0.4	46.1	<0.001
	Control	19.76	11.07	0.7	72.3	

[1]Standard deviation

Table 5. 4. Results of univariable conditional logistic regression model of associations of variables assessed at arrival and mortality ≤21 days after arrival at a milk-fed veal facility for 135 case calves and 270 control calves.

Variable	Description	n	Odds Ratio	95% CI	*P*
Rectal Temp.	< 40°C	377	Ref.		
	≥ 40°C	25	1.85	0.84 to 4.05	0.13
Fecal Score	0 and 1	345	Ref.		
	2	39	1.85	0.90 to 3.83	0.09
	3	19	2.44	0.94 to 6.30	0.07
Navel Score	0 and 1	296	Ref.		
	2	78	1.61	0.93 to 2.77	0.09
	3	31	2.21	1.04 to 4.70	0.04
Dehydration Score	0	201	Ref.		
	1	137	1.88	1.13 to 3.12	0.01
	2	53	2.04	1.00 to 4.19	0.05
	3 and 4	11	5.83	1.58 to 21.41	<0.01
Sunken Flank	Yes	98	Ref.		
	No	305	0.65	0.36 to 1.18	0.16
Source	Local	85	Ref.		
	Drover	269	0.59	0.36 to 0.98	0.04
	Auction	51	0.66	0.30 to 1.48	0.31
Weight	Every 1 lb increase		0.96	0.94 to 0.99	<0.01
IgG	Every 1 g/L increase		0.94	0.91 to 0.96	<0.01
Glucose	Every 1 mmol/L increase		0.77	0.62 to 0.94	0.01
Cholesterol	Every 1 mmol/L increase		0.23	0.13 to 0.39	<0.01

Table 5. 5. Final multivariable conditional logistic regression model describing the associations among significant independent variables and the outcome early mortality (≤ 21 days after arrival at a milk-fed veal facility) using data from 135 case calves and 270 control calves.

Variable	Description	Odds Ratio	95% CI	*P*
Navel Score	0 and 1	Ref.		
	2	2.22	1.10 to 4.50	0.03
	3	2.51	0.98 to 6.41	0.06
Dehydration Score	1	Ref.		
	2	1.36	0.74 to 2.48	0.32
	3	1.16	0.48 to 2.78	0.74
	4 and 5	6.10	1.12 to 33.19	0.04
IgG	Every 1 g/L increase	0.94	0.91 to 0.97	<0.01
Weight	Every pound increase	0.97	0.94 to 1.00	0.04
Cholesterol	Every 1 mmol/L increase	0.28	0.16 to 0.50	<0.01
Source	Local	Ref.		
	Drover	0.48	0.25 to 0.93	0.03
	Auction	0.61	0.20 to 1.86	0.39

Table 5. 6. Cut points calculated by Youden's Index for IgG, weight and cholesterol from blood samples collected from 135 cases and 270 controls at arrival to a milk-fed veal calf facility.

Variable	Cut-point	Se[a] (%)	Sp[b] (%)	AUC[c]
IgG	≥ 16.7 g/L	34	49	0.45
Weight	≥ 102.5 lbs	36	54	0.41
Cholesterol	≥ 1.6 mmol/L	36	40	0.38

[a]Sensitivity: Defined as the proportion of cases (died ≤ 21 days after arrival) having a test result above the cutpoint.

[b]Specificity: Defined as the proportion of controls (survived > 21 days after arrival) having a test result below the cutpoint.

[c]Area under the curve: Probability that a randomly selected case having a greater score than a randomly selected control.

Figure 5. 1. Causal diagram describing the hypothesized relationship of measured variables to mortality occurring during the first 21 days of the growing period at a milk-fed veal calf facility in Ontario.
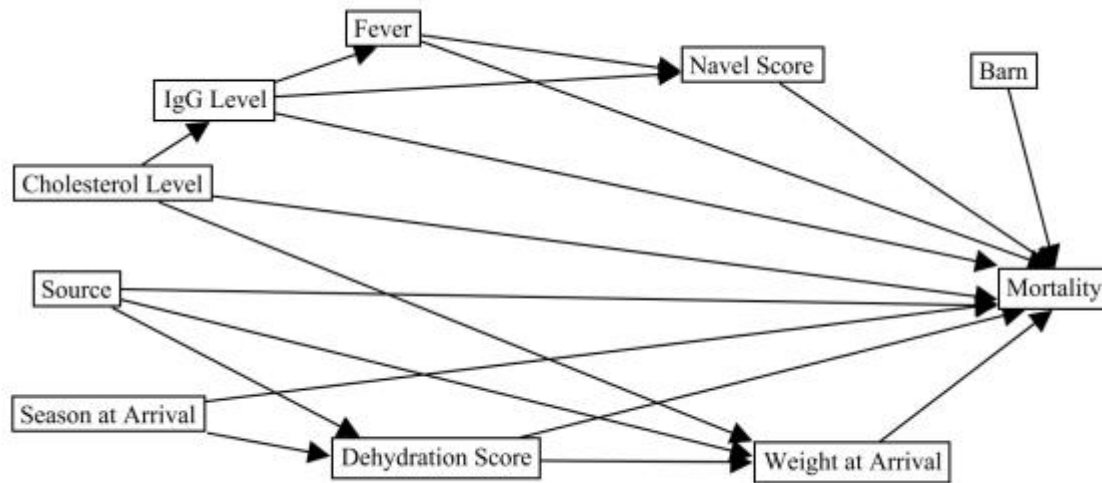
CHAPTER 6: VALIDATION OF COMMERCIAL LUMINOMETRY SWABS FOR TOTAL BACTERIA AND COLIFORM COUNTS IN COLOSTRUM-FEEDING EQUIPMENT

As previously published

VALIDATION OF COMMERCIAL LUMINOMETRY SWABS FOR TOTAL BACTERIA AND COLIFORM COUNTS IN COLOSTRUM-FEEDING EQUIPMENT

ABSTRACT

A sufficient quantity and quality of colostrum must be fed quickly to the newborn calf while minimizing bacterial contamination. Adenosine triphosphate (ATP) bioluminescence swabs offer a potential rapid on-farm alternative to assess bacterial contamination of colostrum. The objective of this study was to validate the Hygiena^{TM} AquaSnap Total (AS), SuperSnap(SS), PRO-Clean (PC) and MicroSnap Coliform (MS) swabs as well as visual hygiene assessment for detection of elevated bacterial counts in or on colostrum-feeding equipment. From April to October 2016, 18 esophageal tube feeders, 49 nipple bottles and 6 pails from 52 dairy farms in Ontario were evaluated for cleanliness. Following visual hygiene assessment, sterile physiological saline (15 ml) was poured into each piece of equipment, mixed for 2 minutes to ensure total surface coverage and poured into a sterile collection container through the feeding end. The fluid was split into equal aliquots, with one being evaluated by conventional culture and the other evaluated using the luminometry swabs. Non-parametric receiver operator curves were used to compare the test performance of the luminescence reading (relative light units (RLU)) from each type of swab to conventional bacterial culture. The area under the curve (AUC) comparing the AS swab to total bacterial count (TBC) (cut point >100,000 colony forming units (cfu)/ml) was 0.89 and using a cut point of 631 RLU correctly classified 84% of samples with a sensitivity of 88% and a specificity of 77%. The AUC comparing the MS swab to total coliform count (cut point >10,000 cfu/ml) was 0.85 and using a cut point of 44 RLU correctly classified 89% of samples with a sensitivity of 83% and a specificity of 90%. Visual hygiene assessment, PC and SS swabs were not reliable indicators for feeding equipment cleanliness. The results suggest that the AS and MS swabs can be used as an alternative to traditional lab bacterial counts to evaluate cleanliness of colostrum-feeding equipment.

Keywords: Calf, Colostrum, Contamination

INTRODUCTION

Colostrum is an integral component of calf health management. Reduced mortality and morbidity (Raboisson et al., 2016; Wells et al., 1996; Windeyer et al., 2014), improved growth (Faber et al., 2005; Furman-Fratczak et al., 2011), earlier age at first calving (Faber et al., 2005) and increased milk production (DeNise et al., 1989; Faber et al., 2005) have all been shown to be

benefits of successful passive transfer of colostral antibodies. Despite the known importance of colostrum, failure of passive transfer (FPT) remains an issue in both female (Beam et al., 2009; Trotz-Williams et al., 2008) and male dairy calves (Pardon et al., 2015). A contributing factor is the cleanliness of colostrum being fed to calves. Bacterial contamination of colostrum, i.e. total bacterial count (TBC) of >100,000 colony forming units (cfu)/ml or total coliform count (TCC) of >10,000 cfu/ml (McGuirk and Collins, 2004; Morill et al., 2012), is common. Contaminated colostrum introduces bacteria that can bind or block IgG absorption (Baintner, 2007; Cummins et al., 2017; Staley and Bush, 1985) and can produce disease (Houser et al., 2008; Godden, 2008), so it is fundamental to feed colostrum with low bacterial counts.

There are several critical control points to prevent contamination of colostrum (Stewart et al., 2005). The feeding process and equipment, whether nipple bottle or esophageal tube feeder, can introduce bacteria to colostrum. Although Stewart et al. (2005) failed to demonstrate a significant difference in bacterial contamination after passing colostrum through a tube feeder, the study was conducted on a dairy farm with excellent sanitation of equipment and concluded that if the feeding equipment is not appropriately cleaned between uses it could represent a source of bacterial contamination for colostrum.

There are several methods used to evaluate the cleanliness of feeding equipment. Visual assessment is a frequently used method, but, it has questionable efficacy to identify bacterial contamination (Malik, 2003; Willis et al., 2007). The most efficacious method to monitor bacterial contamination is through laboratory culture (Godden, 2008; McGuirk and Collins, 2004). The challenge with this method is that the results may not be available for several days, making timely demonstration of a cleanliness problem difficult.

Adenosine triphosphate (ATP) bioluminescence offers a potential rapid on-farm alternative to traditional bacterial culture counts. This technology is well established in the food service industry and its use is rising in the health care industry (Shama and Malik, 2013). As ATP serves as an energy source for all plant, animal, and microbial cells, its presence indicates the presence of organic matter including bacterial contamination (Amodio and Dino, 2014; Finger and Sischo, 2001). Commercially-available ATP swabs contain luciferase, an enzyme that catalyzes a chemical reaction which produces light proportional to the amount of ATP present (Finger and Sischo, 2001). The light intensity is captured by a luminometer that quantifies the intensity in relative light units (RLU) (Shama and Malik, 2013).

The objective of this study was to validate the Hygiena™ AquaSnap Total (AS), SuperSnap (SS), Pro-Clean (PC) and MicroSnap Coliform (MS) swabs, as well as visual hygiene assessment, to detect elevated bacterial counts in colostrum feeding equipment.

MATERIALS AND METHODS

Experimental Design

This cross-sectional diagnostic accuracy study was conducted on dairy farms in Ontario supplying male calves to two veal operations. Each dairy farm was visited once and during the farm visit, feeding equipment that was used to administer colostrum was randomly selected for further evaluation. The equipment was chosen by flipping a coin for each piece of available equipment until only a single piece of equipment had a result of heads. Random selection was done for nipple bottles, pails and esophageal tube feeders if available to a maximum of one per farm of each type of equipment. The surfaces of the equipment that would normally come into contact with the colostrum were scored visually (Table 6.1), receiving a hygiene score of 1 to 4 on the basis of gross contamination of the equipment surface(s) with milk residue or fecal material. Following visual assessment, 15 ml of sterile physiological saline (Vetoquinol™ Lavaltrie, Quebec, Canada) was dispensed into the equipment using a sterile 20 ml syringe. The fluid was gently mixed ensuring coverage of all the surface areas for 2 minutes and then poured into a sterile container (Fisher Scientific™ Waltham, Massachusetts, USA). The container was put immediately into a cooler with ice and transported to the University of Guelph where it was split into equal aliquots, with one submitted to the bacteriology lab at the Animal Health Laboratory for culture and the other evaluated using the luminometry swabs.

Bacterial Analysis

Total bacterial counts (TBC) and total coliform counts (TCC) were performed with the 3M™ Aerobic Colony Count (ACC) Petrifilm (3M™ St. Paul, Minnesota, USA) and 3M™ Coliform Count (CC) Petrifilm (3M™ St. Paul, Minnesota, USA). The wash fluid was diluted serially in 9-ml tubes of phosphate buffered saline to create the following dilutions for ACC: 1:100, 1:1,000, 1:10,000 and 1:100,000 and the following dilutions for CC: neat (undiluted wash fluid), 1:10, 1:100 and 1:1,000. One ml aliquots of each dilution were pipetted onto the respective ACC Petrifilm and CC Petrifilm. The ACC Petrifilm was incubated at 35°C for 48 hours and following incubation, all red-colored colonies were counted to enumerate the TBC. The CC

Petrifilm was incubated at 35°C for 24 hours and following incubation, all red colored colonies with associated gas bubbles were counted to enumerate the total coliform count.

Luminometry Swabs

The PC, SS, AS and MS swabs were used according to the manufacturer's directions (Hygiena[TM] Camarillo, California, USA) on each sample of wash fluid. The product instructions can be found at https://www.hygiena.com/food-and-beverage-atp-tests/.

The Ensure luminometer (Hygiena[TM] Camarillo, California, USA) was used for all swabs except the PC that was assessed based on a color scale (green = clean; grey = caution; light pink = harmful; and dark purple = dangerous) provided with the swab.

Sample Size Calculation

The prevalence of bacterial contamination of colostrum feeding equipment was expected to be 50%. The sensitivity and specificity of the luminometry swabs were estimated to be 71% and 83%, respectively (Finger and Sischo, 2001). Utilizing the method described by Buderer (1996) and assuming the clinically acceptable width of the 95% confidence intervals for sensitivity and specificity was to be no larger than 15%, the sample size required was determined to be 70 samples.

Statistical Analysis

Total bacterial count (TBC) was used as the reference method when evaluating the diagnostic performance of visual hygiene score, SS swab, AS swab, and PC swab and TCC was used as the reference method when evaluating the MS swab and the visual hygiene score. As the $\log_{10}$ bacteria counts were not normally distributed as identified by a significant Shapiro-Wilks test, a Spearman rank correlation coefficient (r) was calculated and a correlation plot was created to describe the relationship between the swabs and the $\log_{10}$ bacteria counts based on the respective reference method. Non-parametric receiver operator curves (ROC) were generated to assess the diagnostic performance when using a cutpoint of 100,000 cfu/ml and 10,000 cfu/ml for TBC and TCC, respectively. Diagnostic test characteristics (sensitivity (Se), specificity (Sp) and predictive values) were calculated at the cut point which correctly classified the most tests. All statistical analyses were performed using STATA 14 (StataCorp LP, College Station, TX).

RESULTS

Descriptive Statistics

A total of 52 dairy farms were visited from April to October of 2016. The participating farms had an average of 135 lactating cows (range: 35 to 520 lactating cows) and 73% housed their lactating cows in free-stall barns. During the visits, 18 esophageal tube feeders, 49 nipple bottles and 6 pails were evaluated. Of the colostrum feeding equipment tested, 41% had a TBC of $\leq$ 100,000 cfu/ml and 79% had a TCC of $\leq$ 10,000 cfu/ml. A descriptive summary of the TBC and TCC is in Table 6.2.

AquaSnap

There was a strong positive correlation between the AS swab RLU and $\log_{10}$TBC (r=0.81; P<0.01; n=73; Figure 6.1). The area under the ROC curve (AUC) (Figure 6.2) was 0.89 (95%CI: 0.82-0.96), correctly classifying 84% of samples at a cut point of 631 RLU. Using this cut-point, Se, Sp and predictive values were calculated (Table 6.3).

SuperSnap

There was no significant correlation between the SS swab RLU and $\log_{10}$ TBC (r=-0.04; P=0.71; n=73). The AUC was 0.49 (95%CI: 0.35-0.64) and using a cut point of 64 RLU, 63% of samples were correctly classified. Se, Sp and predictive values are presented in Table 6.3.

MicroSnap

The MS swab RLU had a moderate positive correlation with the $\log_{10}$TCC (r=0.42; P<0.01; n=73; Figure 6.3). The AUC (Figure 6.4) was 0.85 (95%CI: 0.69-1.00) correctly classifying 89% of samples at a cut point of 44 RLU. The Se, Sp and predictive values using this cut point are in Table 6.3.

Pro-Clean

The PC swab color scale had a negligible negative correlation with the $\log_{10}$TBC (r=-0.19; P=0.12; n=69). The ROC curve generated an AUC of 0.49 and using a cut point of a grey color or greater, only 44% of samples were correctly classified. The Se, Sp and predictive values using this cut point are in Table 6.3.

Visual Assessment

Visual inspections on all farms were completed by a single observer. There was a weak positive relationship between overall hygiene score and $\log_{10}$TBC (r=0.28; P=0.02; n=73). The AUC was

0.61 (95%CI: 0.44-0.79), correctly classifying 71% of samples at a cut point of 3 or greater. Using this cut point, Se, Sp and predictive values were generated (Table 6.3). The correlation between fecal hygiene score and $\log_{10}$TCC was negligible (r=-0.05; P=0.69; n=73) and the AUC was 0.45 (95%CI: 0.33-0.57), correctly classifying 78% of samples at a cut point of 3 or greater. Se, Sp and predictive values were generated using this cut point (Table 6.3).

DISCUSSION

This study demonstrates that bacterial contamination of colostrum feeding equipment is common and that luminometry swabs provide a reasonable alternative to traditional laboratory culture to assess contamination. It also demonstrates that visual hygiene assessment of feeding equipment is a poor indicator of bacterial contamination. The level of bacterial contamination could be over-estimated because the majority of sampling occurred in the summer and season of sampling has been demonstrated to be a risk factor for elevated bacterial contamination of colostrum (Fecteau et al., 2002). Pouring liquid through the colostrum feeding equipment was used to mimic the passage of colostrum and may provide more reliable results when compared to dry swabbing as this method ensures total coverage of the surface area of the feeding equipment. However, there is no literature comparing the 2 methods to evaluate colostrum feeding equipment and more research is necessary to identify the best method for this purpose.

With 59% of feeding equipment wash fluid having >100,000 cfu/ml TBC and 21% having >10,000 cfu/ml, cleanliness of the feeding equipment should be improved. As Stewart et al. (2005) noted, if feeding equipment is not cleaned appropriately it could lead to contaminated colostrum. The proper method to clean feeding equipment is to disassemble equipment, rinse in lukewarm water, place in hot water detergent, scrub all surfaces with a brush, rinse in hot water with acid sanitizer and let dry. If this procedure is followed there should be a limited amount of contamination occurring when colostrum is fed through the equipment (Stewart et al., 2005). It is interesting to note that the nipple bottles and tube feeders had a higher amount of TBC contamination whereas the pails had higher TCC contamination. This could reflect the difficulty in disassembly and cleaning of the nipple bottles and tube feeders and the inability to reach the difficult-to-clean areas. The pails are likely more prone to fecal contamination as the open tops lead to greater chance of manure splashing into the pails.

Visual assessment was an unreliable indicator of feeding equipment cleanliness. When using visual assessment there were many false negatives, demonstrating that many pieces of

equipment that appeared clean were in fact contaminated. These findings are similar to what has been described in hygiene assessment of surfaces in hospitals, where visual assessment was a poor indicator of standards of cleanliness (Cooper et al., 2007; Griffith et al., 2000). As many producers and industry advisors use visual assessment as a method to evaluate hygiene, it is important to dispel this method.

ATP bioluminescence provides a viable alternative to both visual assessment and bacterial culture. The ability to perform the entire process in minutes and immediately demonstrate to producers the level of contamination, make these swabs a practical mechanism to evaluate feeding equipment cleanliness. This technology, despite the initial cost of the luminometer, could be used as part of a calf health monitoring program provided by herd advisors.

The AS and MS performed well when compared to TBC and TCC, respectively. Both swabs had positive moderate to strong correlation to their specific bacteria counts and showed a reasonable performance based on Se, Sp and predictive values. The SS swab did not perform well. The SS had a poor specificity with many of the negative samples having positive test results. To increase the specificity, the RLU cut point could be increased, however this would be at the expense of sensitivity. The main purpose of the PC swab was to detect protein residue as a surrogate measure of surface contamination, and cross-reaction with colostrum and milk residues could explain the swab's overall poor performance in identifying bacterial contamination of colostrum feeding equipment.

The performance of the AS and MS swabs were similar to other studies evaluating ATP bioluminescence as a tool to identify microbial contamination in food stuff such as liquid milk and bottled water (Deininger and Lee, 2001; Luo et al., 2009; Meighan et al., 2014) and hospital equipment (Alfa et al., 2013; Sciortino and Giles, 2012). The reduced level of specificity that was seen with the AS, SS and PC could be due to somatic cells or other non-microbial sources of ATP creating bioluminescence background (Bottari et al., 2015). However, as it is a greater risk not to identify a truly contaminated sample than to have a false positive, the higher levels of sensitivity may overcome the limitation of low specificity.

CONCLUSIONS

A majority of the colostrum feeding equipment evaluated had bacterial contamination above the suggested target of >100,000 cfu/ml TBC and >20% exceeded the target of >10,000

cfu/ml TCC. The AS and MS performed well when evaluating wash fluid from the equipment and provide a rapid on-farm alternative to traditional laboratory methods to quantify the degree of bacterial contamination of colostrum feeding equipment.

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age. Prev. Vet. Med. 113:231–240. http://doi:10.1016/j.prevetmed.2013.10.019.

Table 6. 1.Visual Hygiene Assessment scoring chart used to assess contamination of colostrum feeding equipment

Score	Overall Hygiene Score	Milk/Colostrum	Fecal Material
1	Feeding equipment is visibly clean (ie. No fecal material or milk/colostrum debris residue).	No milk/colostrum debris residue is visible.	No fecal material is visible.
2	Trace amounts of manure and/or milk/colostrum residue are visible.	Trace amounts of milk/colostrum residue are visible.	Trace amounts of manure residue are visible.
3	Manure and/or milk/colostrum residue is clearly visible.	Milk/colostrum residue is clearly visible.	Manure is clearly visible.
4	Manure contamination and/or milk/colostrum residue is extensive.	Milk/colostrum residue is extensive.	Manure contamination is extensive.

Table 6. 2. Descriptive summary of Total Bacterial Count (TBC) and Total Coliform Count (TCC) in colostrum feeding equipment measured using Petrifilm (3M™ St. Paul, Minnesota, USA) in a diagnostic laboratory

Equipment Type		Total Bacterial Count			Total Coliform Count		
		$\leq$100,000	>100,000	>1,000,000	$\leq$10,000	>10,000	>50,000
Nipple	N	19	30	20	39	10	4
Bottle	%	39%	61%	41%	80%	20%	8%
Esophageal	N	5	13	8	15	3	3
Feeder	%	28%	72%	44%	83%	17%	17%
Pail	N	6	0	0	4	2	2
	%	100%	0%	0%	67%	33%	33%
Overall	N	30	43	28	58	15	9
	%	41%	59%	38%	79%	21%	12%

Table 6. 3. Diagnostic test characteristics of luminometry swabs and visual hygiene assessment, relative to bacterial culture using Petrifilm (3M™ St. Paul, Minnesota, USA) in a diagnostic laboratory

Item	Reference Method	TP[3] (%)	Cutpoint	Sensitivity[5] (%)	Specificity[6] (%)	NPV[7] (%)	PPV[8] (%)
AquaSnap	TBC[1]	58.9	631 RLU[4]	88.4	76.7	84.5	82.1
SuperSnap	TBC[1]	58.9	64 RLU[4]	83.7	30.0	56.3	63.2
MicroSnap	TCC[2]	16.4	44 RLU[4]	83.3	90.2	96.5	62.4
Pro-Clean	TBC[1]	57.1	≥Grey	15.0	83.3	40.5	56.5
Overall Visual[9]	TBC[1]	58.9	≥3	30.2	80.0	44.3	68.5
Fecal Visual[10]	TCC[2]	16.4	≥3	0.0	93.4	39.4	0.0

[1]Total bacterial count determined using Petrifilm (3M™ St. Paul, Minnesota, USA) in a diagnostic laboratory.

[2]Total coliform count determined using Petrifilm (3M™ St. Paul, Minnesota, USA) in a diagnostic laboratory.

[3]True prevalence: Defined as the percentage of samples tested with a TBC >100,000 colony forming units/ml or TCC >10,000 colony forming units/ml

[4]Relative light units.

[5]Defined as the probability of testing positive for bacterial contamination using luminometry swabs or visual hygiene assessment, given the feeding equipment has a level of TBC >100,000 colony forming units/ml or TCC >10,000 colony forming units/ml

[6]Defined as the probability of testing negative for bacterial contamination using luminometry swabs or visual hygiene assessment, given the feeding equipment has a level of TBC ≤100,000 colony forming units/ml or TCC ≤10,000 colony forming units/ml

[7]Positive Predictive Value: Defined as the probability the feeding equipment has a level of TBC >100,000 colony forming units/ml or TCC >10,000 colony forming units/ml, given the feeding equipment tested positive for bacterial contamination according to luminometry swabs or visual hygiene assessment

[8]Negative Predictive Value: Defined as the probability the feeding equipment has a level of TBC $\leq$100,000 colony forming units/ml or TCC $\leq$10,000 colony forming units/ml, given the feeding equipment tested negative for bacterial contamination according to luminometry swabs or visual hygiene assessment

[9] Overall hygiene score created through visual assessment of feeding equipment (Table 1)

[10] Fecal material score created through visual assessment of feeding equipment (Table 1)

Figure 6. 1. Relationship between $\log_{10}$ total bacterial count (TBC) determined by Petrifilm (3M[TM] St. Paul, Minnesota, USA) and relative light units (RLU) determined by a commercial luminometry assay (AquaSnap (Hygiena[TM] Camarillo, California, USA)
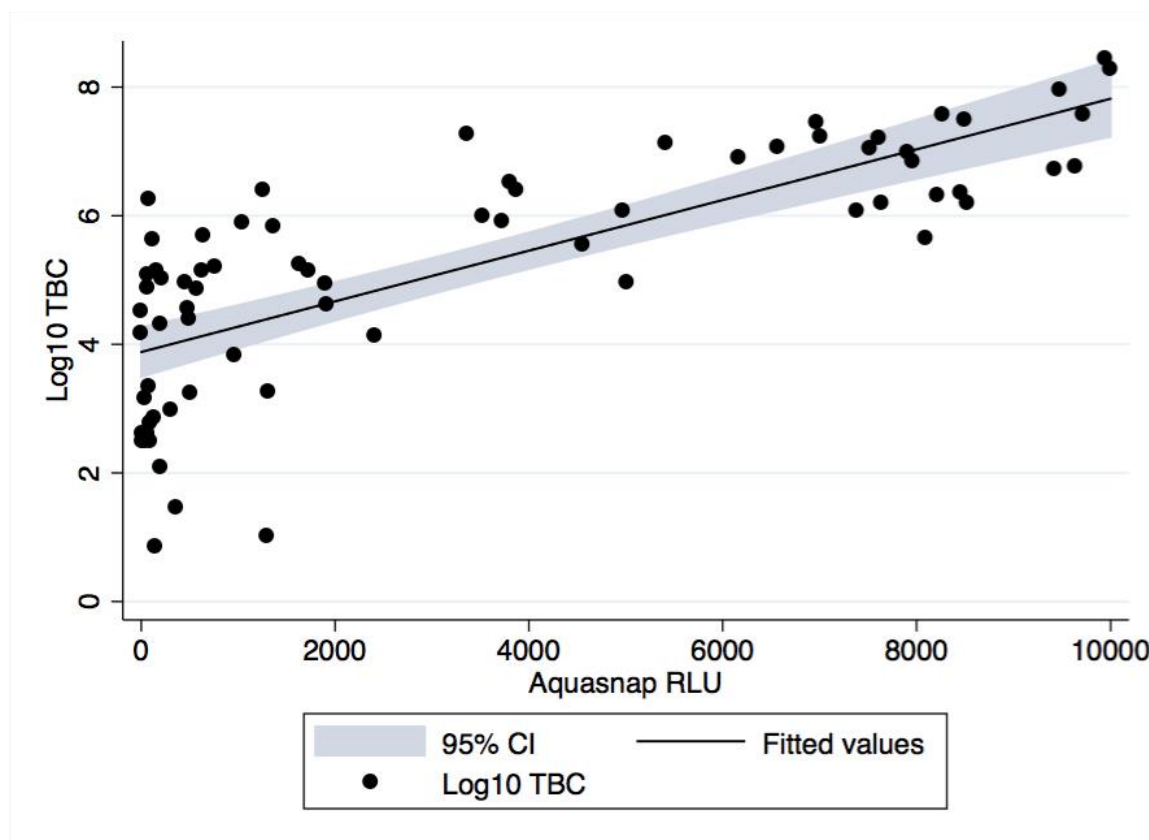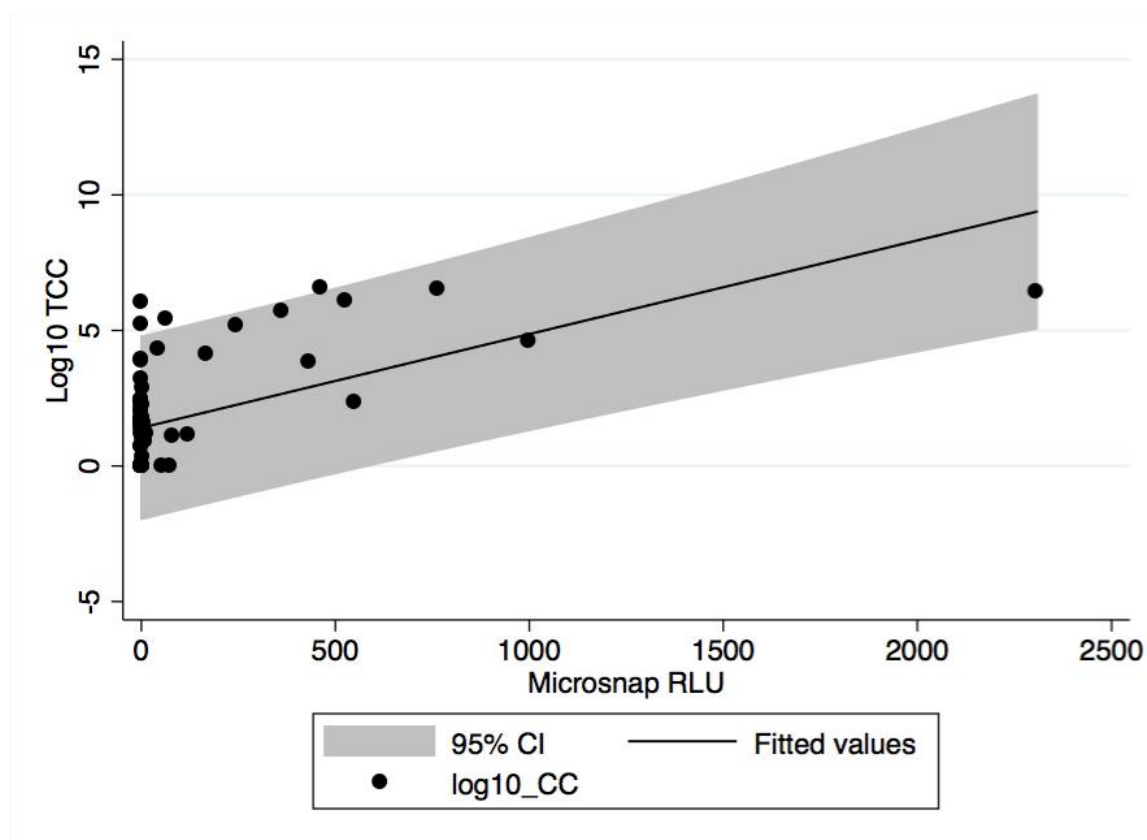
Figure 6. 2. Receiver Operator curve comparing relative light units determined by a commercial luminometry assay (AquaSnap (Hygiena[TM] Camarillo, California, USA) to total bacterial count (TBC) determined by Petrifilm (3M[TM] St. Paul, Minnesota, USA)
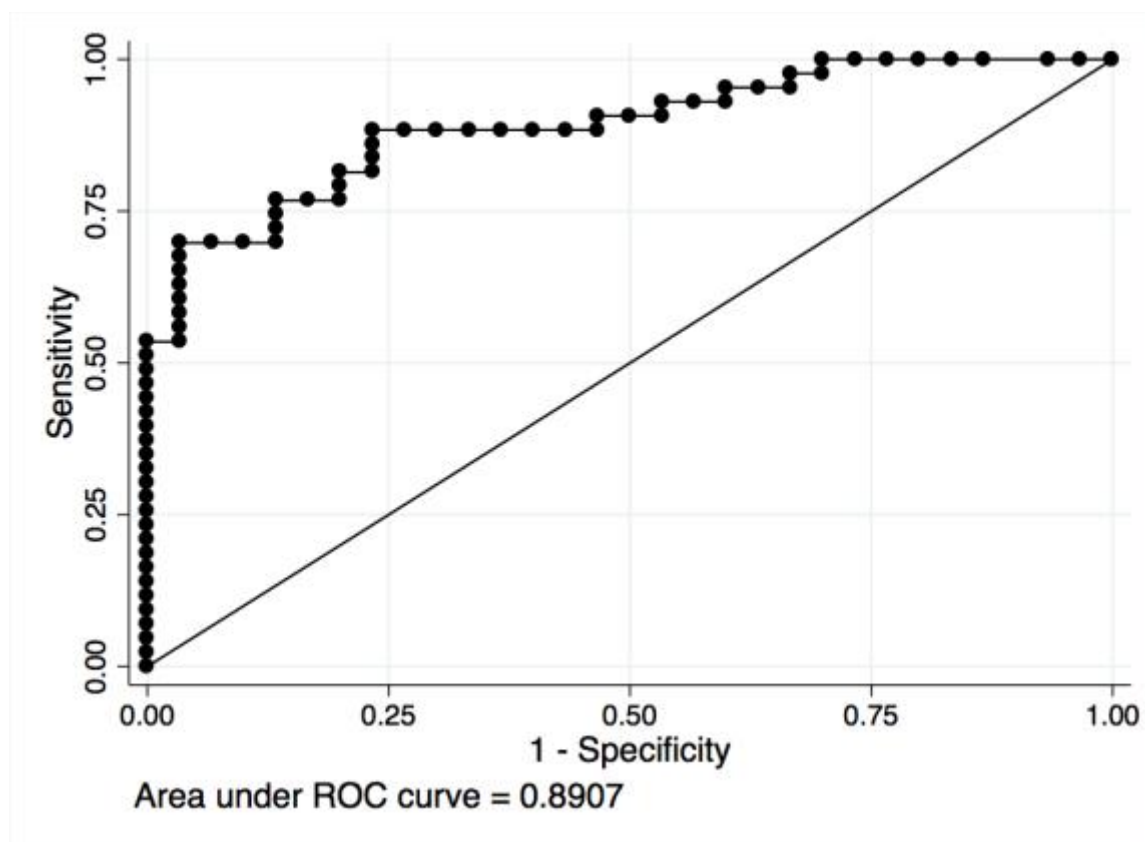
Figure 6. 3. Relationship between $\log_{10}$ total coliform count (TCC) determined by Petrifilm (3M^{TM} St. Paul, Minnesota, USA) and relative light units (RLU) determined by a commercial luminometry assay (MicroSnap (Hygiena^{TM} Camarillo, California, USA)

Figure 6. 4. Receiver Operator curve comparing relative light units determined by a commercial luminometry assay (MicroSnap (Hygiena[TM] Camarillo, California, USA) to total coliform count (TCC) determined by Petrifilm (3M[TM] St. Paul, Minnesota, USA)

Area under ROC curve = 0.8511
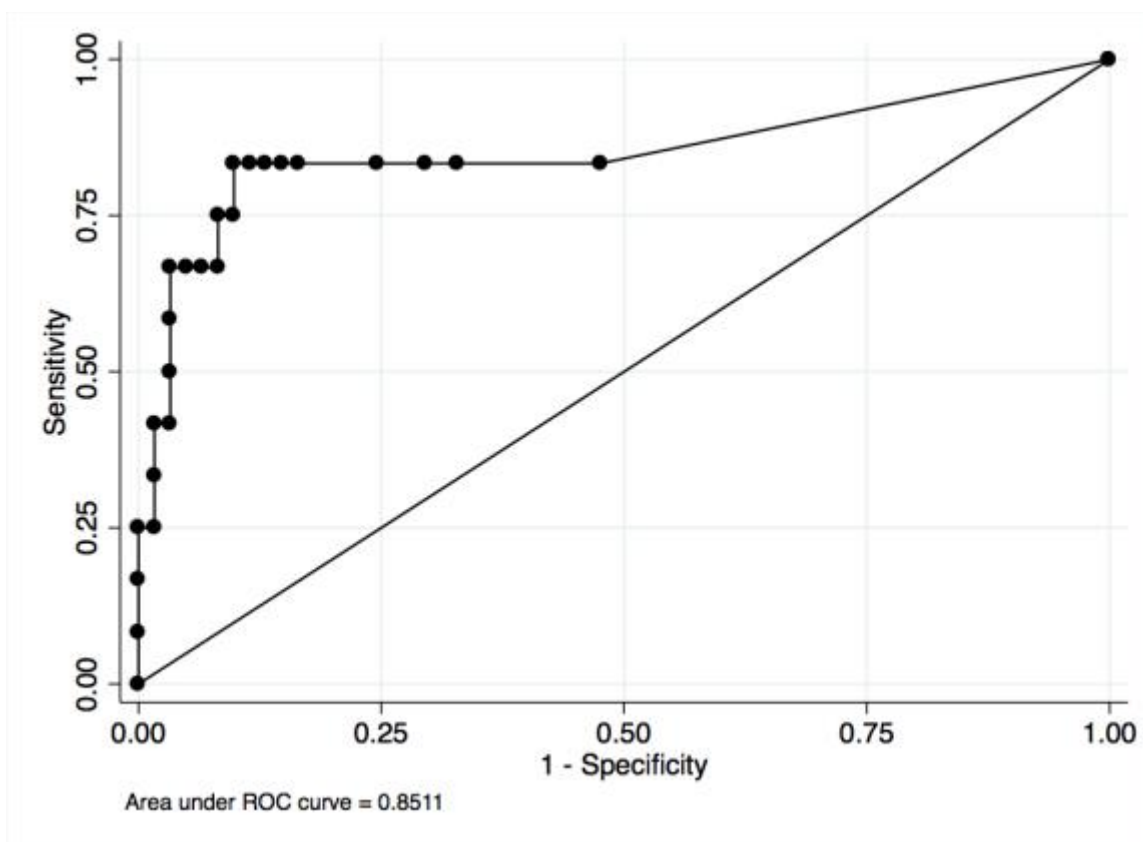
CHAPTER 7

GENERAL CONCLUSIONS AND LIMITATIONS

The purpose of the research studies described in this thesis were to explore risk factors impacting male dairy calf health at the source dairy farms and upon arrival at a veal facility. In its totality, this work demonstrated that although most dairy producers treat male calves well, and similarly to female dairy calves, a significant number of calves are entering into the veal industry with health abnormalities that impact their short and long-term well-being.

The first objective, explored in Chapter 2, was to describe management practices associated with the care of male calves during their first days of life on Canadian dairy farms. This was the first study to evaluate the care of male calves at source dairy farms. A small proportion of producers (9%) did not always feed colostrum to male calves and 17% did not feed male calves the same as females. With FPT (Pardon et al., 2015; Trotz-Williams et al., 2008; Wilson et al., 2000) and emaciation (Wilson et al., 2000) being commonly identified in calves arriving at veal facilities, improving colostrum management and nutrition could have serious welfare implications and affect the incidence of disease occurring in the veal industry (Pardon et al., 2015; Todd et al., 2017). Geographical region, herd size and familiarity with the code of practice were variables associated with many aspects of the management of male calves on dairy farms; however, as the survey covered superficial characteristics of the dairy farms, it was difficult to further explore the reasons for these associations. Although there were some questionable practices performed by dairy producers, most treated male calves well, which challenges some speculative statements made by Bähler et al. (2012) and Schnepper (2001).

As with most volunteer based survey's, it is likely that the results presented in Chapter 2 were subject to some selection bias. With the response proportion being 9% of all Canadian producers, non-response bias may have influenced the results, with producers milking more cows and housing their cattle in free-stalls being more likely to respond to the survey. Hence, the responses may not be completely reflective of the entire Canadian dairy industry. The Hawthorne effect (McCambridge et al., 2014), where individuals under observation modify an aspect of their behavior, may also have introduced bias. This may have had an effect when covering more controversial aspects of the survey such as euthanasia practices and feeding colostrum to male calves. Despite the potential biases present, Chapter 2 provides insight into the management of

male calves on dairy farms creating potential avenues to improve certain management practices and ultimately advance the health and welfare of male calves.

The second objective was to assess the association between calf management practices on source dairy farms and mortality occurring on veal farms. In Chapter 3, a cross-sectional study explored this objective. This is the first study to trace male calves back to the source dairy farms to explore this question. Based on the results of this study, bedding type, feeding method used for colostrum, veterinarian not inquiring about the health and performance of calves during regular herd health visits and the frequency of observation of the calving area during the day were associated with high mortality source dairy farms. With the association between neonatal care and health being well demonstrated in female dairy calves (Gulliksen et al., 2009; Wells et al., 1996; Windeyer et al., 2014), it is not surprising that some practices on source dairy farms impact health at the veal farm. With veal producers relying on dairy producers to provide the necessary care of newborn male calves, the collaboration between these two industries needs to continue to grow in order to improve the health and welfare of male calves.

The relatively small number of source dairy farms visited in Chapter 3 was one of the limitations of the study. This reduced power, may have created exaggerated estimates of the magnitude of effects and likely resulted in more type 2 errors (Button et al., 2013). Similar to Chapter 2, the Hawthorne effect likely influenced the outcomes. As the dairy producers recognized that the purpose of the project was to study the health of male calves, their answers to the questionnaire may have been altered to reflect idealistic management of male calves. Non-response bias could also have been introduced in this study as it relied on volunteers for participation. However, with little information about the non-responders it is difficult to imply that this type of selection bias occurred. Another potential source of error could be found on source dairy farms that shipped a smaller quantity of calves, a high amount of weight was placed on each individual calf with the logistic model that was used.

In Chapter 4 and 5, the objectives were to describe the health status of calves upon arrival at a veal facility, and to associate characteristics of the arriving calf with early and late mortality. Other studies have compared health status at arrival with morbidity (Wilson et al., 2000) and mortality (Bähler et al., 2012), however, this is the first to evaluate the effects in Canada. Overall, a significant number of calves entered the veal facility with a health abnormality. This is a clear welfare concern and the findings were similar to other published studies (Bähler et al.,

2012; Pempeck et al., 2017; Wilson et al., 2000), demonstrating that this issue may be ubiquitous. Many of these health abnormalities were associated with early and late mortality, including navel score, cough score, dehydration level, presence of a sunken flank and weight at arrival. The level of immunoglobulin G and cholesterol were the metabolic parameters associated with early mortality. With the high levels of health abnormalities and their association with mortality, measures need to be implemented to prevent these from occurring, prior to entering the veal facility.

The main limitation present with the work presented in these two chapters was the subjective nature of the scoring system used, and the multiple observers that scored the calves. This may have created a form of non-differential misclassification of exposure, biasing the results towards the null. The milk-fed veal facility also did not adequately record the therapies given to the calves and the inability to control for the therapies would influence the ability of the calf to survive, however, this would have been an intervening variable in the analysis. If treatments were recorded accurately, it would have allowed further exploration regarding antibiotic use in this facility and the major factors influencing it.

The objective of Chapter 6 was to validate commercial luminometer swabs for the detection of elevated bacterial counts in or on colostrum feeding equipment. From a descriptive standpoint, the feeding equipment was grossly contaminated with 59% and 38% having a total bacterial count > 100,000 cells/ml or > 1,000,000 cells/ml, respectively. With colostrum having high levels of contamination (Morill et al., 2012), the high levels of bacterial contamination in the colostrum feeding equipment would likely have a significant influence on bacterial counts in colostrum. However, the levels found on these farms may not be representative of the dairy industry as it was not a random subset and the farms visited were, on average, larger than Canadian dairy farms. The work described in this chapter also demonstrated the utility of this instrumentation as a potential on-farm tool to quickly demonstrate to producers that their feeding equipment was contaminated. It also dispelled visual hygiene assessment as a method to detect bacterial contamination, which is consistent with conclusions described in other studies evaluating hygiene in human hospitals (Malik, 2003; Willis et al., 2007).

FUTURE WORK

Male calf mortality ranges from 4% to 8% (Bähler et al., 2012; Winder et al., 2016) based on estimates published in peer-reviewed literature, and with health and welfare being important to support the sustainability of the veal industry, action must be taken to reduce mortality levels. The work described in this thesis identifies several risk factors associated with mortality and could focus attention on some key areas for intervention, and more importantly prevention of disease in male calves.

With many producers relying on oral antibiotic treatment at arrival at the veal farms (Pardon et al., 2012), selective antibiotic treatment could be explored as a method to drastically reduce the quantity of antibiotics provided to the calves and tailor specific treatment to calves with health abnormalities. Chapter 4 and 5 provide some health parameters to identify high-risk calves that can be evaluated quickly and easily by producers within the veal industry. A randomized clinical trial could be designed to treat solely the high-risk calves with antibiotics and/or supportive therapy as a prudent antimicrobial strategy. With the veal industry being targeted for high-levels of antibiotic use and resistance (Pardon et al., 2014), this could provide a route to overcome this challenge. The impact of health status at arrival on growth and economics should also be explored. This could provide veal producers with a further understanding of the consequences of purchasing a calf with certain health aliments and could provide a potential payment scheme where calves could be deducted or a premium paid based on what is discovered on the exam of the calf.

In Chapter 2, one of the unanswered questions that remained was the reasons why some dairy producers treat male calves differently. Factors influencing dairy producer's attitudes toward male calves need to be explored to determine measures to improve their treatment when on the dairy farms. An incentive based pay scale could be explored to motivate producers with premiums being paid for calves with successful passive transfer or source farms with low mortality. Within the same project, benchmarking source dairy farms TP status and mortality against their peer dairy farms could also be explored as a motivator for improved management.

With a significant amount of uncertainty surrounding the transportation of young calves, further research is warranted. This is especially true regarding age at transportation. A study evaluating calves randomly allocated to different ages of transport may provide insight into this

controversial issue. The type and design of the truck and/or trailer facilitating the transport may also be another potential avenue to explore to reduce transport stress.

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APPENDIX

CHAPTER 3: SOURCE DAIRY FARM QUESTIONNAIRE

Q1 What is the farm name?

Herd Demographics

Q2 What is the barn type?
○ Tie-stall
○ Free-stall
○ Bedded pack
○ Other _____

Q3 Herd Demographics
_____ How many cows are you currently milking?
_____ How many cows did you calve out in the past year?

Calving Area

Q4 Where do calving's occur? (Indicate proportion if more than one system used)
_____ Individual calving pen
_____ Group calving pen
_____ Regular tie stall with covered gutter
_____ Regular tie stall
_____ Regular free stall
_____ Other

Q5 What percentage of calvings occur in the designated calving area?
_____ Percent

Q6 Which option corresponds best to the management of your calving area?
○ Bedding is always changed between calvings
○ Bedding is not systematically changed between calvings but bedding is added and placenta and excretions are removed
○ Bedding is not systematically changed between calvings and no new bedding is added but placenta and excretions are removed
○ No calving area cleaning strategy
○ Changes depending on season _____
○ Other _____

Q7 When you clean the bedding from the calving area, do you wash (soap) or disinfect the calving area?

❍ Yes to wash and disinfect

❍ Wash

❍ Disinfect

❍ No

Q8 Do you use the calving area (pen or stall) for sick or lame cows?

❍ Yes

❍ No

> Display This Question:
>
>   If Do you use the calving area (pen or stall) for sick or lame cows? Yes. Is Selected

Q9 How frequently do you use the calving area for sick or lame cows?

❍ Always

❍ Most of the time

❍ About half the time

❍ Sometimes

Calving Monitoring

Q10 How frequently is the calving area/close up pen checked (includes live or via camera) for cows starting to calve during the day (between morning and evening milking)? (Example: every 4 hours)
_____ Hours

Q11 How frequently is the calving area/close up pen checked (includes live or via camera) for cows starting to calve during the evening (between evening and morning milking)? (Example: every 4 hours)
_____ Hours

Q12 Do you use calving monitoring equipment?
❍ Yes
❍ No

Q13 What equipment do you use?
❍ Video camera
❍ Intravaginal devices
❍ Both
❍ Other _____

Q14 How many people working on the farm assist with calvings?

Q15 What percentage of calvings are observed?
_____ Percent

Q16 Of the calvings that you observe, when do you intervene to assist a heifer calving?
❍ Routinely assist all calvings
❍ After checking and finding a problem
❍ Every time a primiparous cow was calving
❍ Routinely at night or when no staff available for several hours
❍ When calf expected is of high value
❍ When twins suspected
❍ Other _____

Q17 What percentage of heifers are assisted (applying traction or pulling)?
_____ Percent

Q18 What percent of cows are assisted (applying traction or pulling)?
_____ Percent

Q19 If unable to re-position or deliver a calf, what do you do?

Newborn Calf Care

Q20 Do you regularly perform umbilical disinfection/navel dip or spray on your farm?
❍ Yes
❍ No

Display This Question:
    If Do you regularly perform umbilical disinfection on your farm? Yes Is Selected
Q21 Which product and concentration is used for umbilical disinfection?

Display This Question:
    If Do you regularly perform umbilical disinfection on your farm? Yes Is Selected
Q22 When is the product applied after birth?
_____ Hours after birth

Display This Question:
    If Do you regularly perform umbilical disinfection on your farm?; Yes Is Selected
Q23 Does this practice differ between heifer and bull calves?
❍ Yes _____
❍ No

Q24 Is calf vigor evaluated at birth? (Weak, unable to stand, breathing problems, no suckle reflex)
❍ Yes
❍ No

Display This Question:
    If Is calf vigor evaluated at birth? (Weak, unable to stand, breathing problems, no suckle reflex) Yes Is Selected
Q25 How do you use this information?

Display This Question:
    If Is calf vigor evaluated at birth? (Weak, unable to stand, breathing problems, no suckle reflex) Yes Is Selected
Q26 Would it differ if it was a bull vs a heifer calf?
❍ Yes _____
❍ No

Q27 Is discharge from the nose and mouth removed with a clean towel at birth?
○ Yes
○ No

Q28 Are weak calves stimulated at birth during both winter and summer? (For example: rubbing, straw in nose)
○ Yes
○ No

Q29 How are the calves stimulated? Is it different in the winter or summer?

Q30 Do you ever suspend newborn calves by their rear legs?
○ Yes
○ Sometimes (describe when) _____
○ No

Q31 Are calves placed in sternal recumbency upon birth? (sitting upright on stomach with legs placed under their body)
○ Always
○ Sometimes (describe when) _____
○ Never

Q32 Following birth, do you dry the calf?
○ Always
○ Sometimes (describe when) _____
○ Never

Q33 Does this differ in winter vs summer?

Q34 Where is the calf placed following calving?
○ Remains in calving pen with bedding added
○ Remains in calving pen with no bedding added
○ Remains in calving pen but placed into a tub
○ Moved away from the calving pen

Q35 What type of bedding is added?
- ○ Deep bedded straw
- ○ Wood shavings
- ○ Other _____

Q36 Where is the calf moved to?
- ○ Another pen with dam
- ○ Individual calf pen
- ○ Group calf pen

Q37 If the calf tub is used in the calving pen, how often is it cleaned?
- ○ After every calf
- ○ Every other calf
- ○ Every 3-4 calves
- ○ Every 5-10 calves
- ○ Greater than 10 calves

Q38 Does this differ between bull and heifer calves
- ○ Yes (describe) _____
- ○ No

Q39 How long do the calf and dam remain in physical contact after calving?
_____ Hours after calving

Colostrum Feeding Practices

Q40 From the last 10 heifers calves born, how many received colostrum?
_____ Number of heifer calves who received colostrum

Q41 From the last 10 bull calves born, how many received colostrum?
_____ Number of bull calves who received colostrum

Q42 What best describes the colostrum source that is used for heifer calves? (Indicate proportion if more than one system used)

_____ Fresh

_____ Refrigerated

_____ Frozen

_____ Colostrum replacer

Q43 What best describes the colostrum source that is used for bull calves? (Indicate proportion if more than one system used)

_____ Fresh

_____ Refrigerated

_____ Frozen

_____ Colostrum replacer

Q44 If colostrum replacer is used, what quantity of IgG is present and how much is fed?

Q45 How do you decide which source of colostrum to feed?

Q46 When is colostrum fed for the first time to heifer calves? (Indicate proportion if more than one time frame used)

_____ Within 2 hours of birth

_____ Within 2-6 hours after birth

_____ Within 6-12 hours after birth

_____ Greater than 12 hours after birth

_____ Never

Q47 When is colostrum fed for the first time to bull calves? (Indicate proportion if more than one time frame used)

_____ Within 2 hours of birth

_____ Within 2-6 hours after birth

_____ Within 6-12 hours after birth

_____ Greater than 12 hours after birth

_____ Never

Q48 What quantity of colostrum is provided at the first feeding? (Describe: Show bottle, hygiene score bottles)

Q49 Does the quantity of colostrum provided at the first feeding differ between bull and heifer calves?

○ Yes (describe) _____

○ No

Q50 Is a second feeding of colostrum provided within the first 12 hours after birth?

○ Yes

○ No

Q51 What percentage of bull and heifer calves receive a second feeding of colostrum in the first 12 hours after birth?
_____ Heifer calves
_____ Bull calves

Q52 What quantity is given at the second feeding? Does this differ between bull and heifer calves?

Q53 What total quantity of colostrum is fed in the first 24 hours after birth?

Q54 Is the colostrum provided at the first and second feedings always collected from the first milking after calving?
❍ Yes
❍ No

Q55 Does this differ between bull and heifer calves?
❍ Yes (describe) _____
❍ No

Q56 Do you feed transition milk to calves?
❍ Yes
❍ No

Q57 How long do you provide transition milk (milk from second, third and fourth milking after calving) to calves?

Q58 Which method is used to feed colostrum to heifer calves for the first feeding? (Indicate proportion if more than one system used)
_____ Provide colostrum in a pail
_____ Provide colostrum in a bottle fitted with a nipple
_____ Provide colostrum by an esophageal feeder
_____ Calf is left with the dam; no intervention
_____ Calf left with the dam but staff intervention to ensure adequate suckling

Q59 Which method is used to feed colostrum to bull calves for the first feeding? (Indicate proportion if more than one system used)

_____ Provide colostrum in a pail

_____ Provide colostrum in a bottle fitted with a nipple

_____ Provide colostrum by an esophageal feeder

_____ Calf is left with the dam; no intervention

_____ Calf is left with the dam but staff intervention to ensure adequate suckling

Q60 If calves do not drink a sufficient quantity of colostrum spontaneously do you use an esophageal feeder?

○ Yes

○ No

Q61 Does this between bull and heifer calves?

○ Yes (describe) _____

○ No

Q62 Is the feeding method always cleaned (washed with detergent) prior to providing colostrum?

○ Yes

○ No

Q63 Does this differ between bull and heifer calves?

○ Yes (describe) _____

○ No

Colostrum Collection and Storage

Q64 How long after calving until colostrum is collected from a fresh cow/heifer?

_____ Hours after calving

Q65 Which hygiene measures do you apply to colostrum collection? (Check all that apply)

❑ Hands are washed prior to milking

❑ Gloves are worn during milking

❑ Cows teats are cleaned prior to milking

❑ Bucket used to collect colostrum has been cleaned with soap prior to milking

❑ None of the above

Q66 Is colostrum heat treated/pasteurized?

○ Heat-treated

○ Pasteurized

○ Neither

Q67 What setting is used on the pasteurizer? (temperature setting)

Q68 How long is the time period between colostrum milking and either feeding or refrigeration/freezing of colostrum?
_____ Hours

Q69 If refrigerated colostrum is used, do you add in a preservative?
○ Yes
○ No

Q70 What product?

Q71 How long is refrigerated colostrum kept in the fridge for?
_____ Days

Q72 How long is frozen colostrum kept in the freezer for?
_____ Months

Q73 Do you ever pool colostrum from more than one cow prior to storage?
○ Yes
○ No

Q74 Are colostrum containers marked with the date of collection when stored in a fridge or freezer?
○ Yes
○ No

Q75 Describe how colostrum is warmed from frozen or refrigerated.

Q76 Do you always have sufficient colostrum reserves for 2 calves in the freezer?
○ Yes
○ No

Q77 Do you assess colostrum quality? (visually = no)
○ Yes
○ No

Q78 How do you assess colostrum quality?
❍ Brix refractometer
❍ Colostrometer
❍ Other _____

Q79 Are blood samples taken from calves to evaluate passive transfer?
❍ Yes
❍ No

Q80 Which calves do you sample?
❍ Bulls
❍ Heifers
❍ Both

Calf Housing

Q81 What type of housing is used for pre-weaned calves?
❑ Individual pens indoors
❑ Individual hutches outdoors
❑ Group pens indoors
❑ Group pens outdoors
❑ Other _____

Q82 Does this differ between winter and summer?
❍ Yes _____
❍ No

Q83 Does this differ between bull and heifer calves?
❍ Yes (describe) _____
❍ No

Q84 At what age do you introduce group housing to calves?
_____ Weeks of age

Q85 How often is bedding added to calves' housing?
❍ Twice per week or more
❍ Once per week
❍ Less than once per week

Q86 Does this differ between bull and heifer calves?
- ○ Yes (describe) _____
- ○ No

Q87 How often is bedding completely cleaned out from calves' housing?
- ○ At least once per week
- ○ Once every 2 weeks
- ○ Once every 4 weeks
- ○ Less than once per month
- ○ No bedding

Q88 Does this differ between bull and heifer calves?
- ○ Yes (describe) _____
- ○ No

Q89 When you remove bedding from calves' housing, do you wash (with soap) or disinfect?
- ○ Yes
- ○ No

Q90 Does this differ between bull and heifer calves?
- ○ Yes (describe) _____
- ○ No

Q91 What kind of ventilation system is used in calf housing?
- ○ Open, natural
- ○ Positive pressure ventilation
- ○ Fans
- ○ Other _____

Q92 What type of bedding is used? (Check all that apply)
- ❑ Long straw
- ❑ Chopped straw
- ❑ Sand
- ❑ Shavings
- ❑ Other _____

Q93 Does this differ between bull and heifer calves?
- ○ Yes (describe) _____
- ○ No

Q92 How long are bull calves kept on farm for?
- ❍ 1-3 days
- ❍ 4-7 days
- ❍ 8-10 days
- ❍ >10 days
- ❍ Whenever they are picked up

Q94 How frequently are bull calves picked up?

Q95 What criteria do you use to decide if a bull calf is fit to leave the farm? Describe.

Calf Feeding

Q96 How much milk or milk replacer is provided to bull calves per day (in L)?
- ❑ 1st week of life _____
- ❑ After 1st week _____

Q97 How much milk or milk replacer is provided to heifer calves per day (in L)?
- ❑ 1st week of life _____
- ❑ After 1st week _____

Q98 What method do you use to feed milk or milk replacer to bull calves?
- ❑ Bottle with nipple
- ❑ Nipple pail
- ❑ Open pail
- ❑ Automatic delivery system
- ❑ Large nipple bucket (mob feeder)
- ❑ Other _____

Q99 What method do you use to feed milk or milk replacer to heifer calves?
- ❑ Bottle with nipple
- ❑ Nipple pail
- ❑ Open pail
- ❑ Automatic delivery system
- ❑ Large nipple bucket (mob feeder)
- ❑ Other _____

Q100 How often are the feeding utensils (buckets, bottles, nipples, mixing tools) cleaned for bull calves?

❍ Daily
❍ Bi-weekly
❍ Monthly
❍ Between calves
❍ Never

Q101 How often are the feeding utensils (buckets, bottles, nipples, mixing tools) cleaned for heifer calves?
❍ Daily
❍ Bi-weekly
❍ Monthly
❍ Between calves
❍ Never

Q102 How are they cleaned? Describe.

Q103 What are pre-weaned bull calves fed? (Indicate proportion if more than one system used)
_____ Commercial milk
_____ Waste milk (milk from cows under antibiotic treatment or in the withdrawal period)
_____ Milk replacer
_____ Other

Q104 What are pre-weaned heifer calves fed? (Indicate proportion if more than one system used)
_____ Commercial milk
_____ Waste milk (milk from cows under antibiotic treatment in the withdrawal period)
_____ Milk replacer
_____ Other

Q105 Do you pasteurize or acidify the milk that will be fed to unweaned calves?
❍ Yes
❍ No

Animal Monitoring

Q106 Do you keep a written record of all disease cases (clinical signs and treatments administered) as well as calf and heifer mortality between birth and first calving?
❍ Yes
❍ No
❍ Sometimes _____

Q107 Do you have written treatment protocols for calf illness?
❍ Yes
❍ No

Veterinary Assistance

Q109 How often is a veterinarian present on the farm?
- ◯ Every week
- ◯ Every two weeks
- ◯ Every month
- ◯ More than every month
- ◯ Never

Q110 Does your veterinarian ask about the health or performance of the calves?
- ◯ Always
- ◯ Sometimes
- ◯ Seldom
- ◯ Never